

https://dspace.org/

An epidemiological study of multiple myeloma in Southern Africa

Patel, Moosa

2014-03-20
http://hdl.handle.net10539/14255

Downloaded from a DSpace repository. https://dspace.org/

AN EPIDEMIOLOGICAL STUDY OF MULTIPLE MYELOMA IN SOUTHERN AFRICA

Moosa Patel

A thesis submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD).

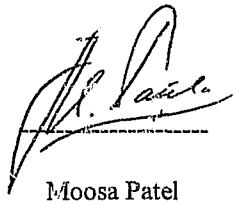
Johannesburg, 1999.

ETHICS COMMITTEE APPROVAL

This research was approved by the Ethics Committee for Research on Human Subjects, University of the Witwatersrand (Clearance Certificate Numbers: 09/11/92 and 10/11/92).

DECLARATION

I declare that this thesis, apart from some technical and routine laboratory investigations performed on the blood and bone marrow of the patients included in this study, is my own unaided work. It is being submitted for the degree of Doctor of Philosophy to the University of the Witwatersrand. It has not been submitted before for any degree or examination at this or any other University.



Moosa Patel

19/11/99

Date

DEDICATION

To my parents, wife and four sons

PUBLICATIONS AND PRESENTATIONS

1. PRESENTATIONS

Patel M., Celliers L., Wadee A.A., Mendelow B.V. and Galpin J.S. Human Leukocyte Antigens and Multiple Myeloma. Proc Future Path Congress, 1998.

Patel M., Philip V., Wadee A.A., Gavalakis C., Mendelow B.V., Fourie A.M. et al. Multiple Myeloma Associated with Class I and Class II Human Leukocyte Antigens. Proc SASMO/SASRO Congress, 1998.

Patel M., Mahlangu J., Mendelow B.V., Patel J.B., Stevens G. and Stevens W. Kaposi's Sarcoma-Associated Herpesvirus infection/Human Herpesvirus -8 (HHV-8) infection and Multiple Myeloma. Proc SASMO/SASRO Congress, 1998.

Patel M., Mahlangu J., Patel J.B., Stevens G., Stevens W., Allard U. and Mendelow B.V. Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 and multiple myeloma in southern Africa. VIIth International Myeloma Workshop, Sweden, 1-5 Sept. 1999.

2. PUBLICATIONS

Patel M., Mahlangu J., Patel J.B., Stevens G., Stevens W., Allard U. and Mendelow B.V. Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 (KSHV/HHV-8) and multiple myeloma in southern Africa. Diagnostic Molecular Pathology (in press).

ABSTRACT

Globally, there are both striking geographic and ethnic differences in myeloma incidence. Myeloma is twice as common in American blacks compared to caucasians. The age-adjusted incidence rates for caucasians is 4.1/100 000 and for blacks is 9.1/100 000 (Ries et al, 1994). The higher rate of myeloma in blacks is not confined to the USA, but may also include other regions of the world. Blattner et al (1979), found that a similarly high rate of myeloma was present in South African blacks. Despite having made this important observation two decades ago, there have been no epidemiological studies in South Africa, and indeed in Africa, that have explored the role of the potential and suspected risk factors that are implicated in the aetiopathogenesis of the disease. This thesis was designed to address this deficiency and fill the gap that exists.

This thesis examines the role of some of the suspected risk factors in the aetiopathogenesis of myeloma, in a population of patients in whom myeloma is the commonest lymphohaematopoietic malignancy. The influence of occupational, sociodemographic and other characteristics were evaluated using a case-control study. Environmental factors such as Kaposi's sarcoma associated herpesvirus/human herpesvirus-8 (KSHV/HHV-8) were indirectly evaluated by detecting the presence of KSHV DNA sequences in bone marrow aspirates, bone marrow biopsy material and/or cultured bone marrow adherent cells, using a nested PCR (polymerase chain reaction) assay. In addition, class I and class II HLA (Human Leucocyte Antigen) types were identified in the myeloma patients and compared to an ethnically matched control population. Finally, the flow cytometric characteristics of myelomatous plasma cells

including DNA ploidy analysis and relevant plasma cell antigen expression were determined. The clinical profile of the background myeloma population in whom these studies were conducted was documented. This, together with the relevant prognostic factors is presented and compared to the findings in Africa and the Western world.

A total of 170 patients with myeloma were seen from January 1992 to December 1997. This constitutes the background myeloma population in this study. Initially, two aspects of the study were undertaken, viz., the case-control study and the flow cytometry study. Interviews were possible in 130 of the 170 patients. The other 40 patients were not interviewed for primarily two reasons. Firstly, a number of patients died during the initial admission and secondly, patients who were not interviewed during the first admission did not return for follow up (were lost to follow up). Similarly, flow cytometry was possible in 103 of the 170 patients. The reduced numbers were due to the following reasons:

i) some patients had bone marrow aspirates performed at a peripheral hospital or by a private physician prior to being referred to our hospital and the bone marrow was not repeated by us and ii) for technical reasons a flow cytometric analysis was not possible - insufficient or grossly haemodilute specimen, and in some instances there was a dry tap.

The HLA study was commenced in 1995, by which time a number of the original patients had died and some were lost to follow up. Sixty two patients had HLA studies performed (57 of whom were part of the 170 patients). Towards the latter part of this 'epidemiological study', important information regarding the role of KSHV and myeloma emerged. A brief analysis regarding this association was deemed pertinent and was

included prior to concluding the thesis. Thus, the numbers represented in this aspect of the study are much smaller (25 of the 170 patients).

The median age at presentation of 61.4 years in our patients is intermediate between the higher figures quoted for patients in the Western world, and the lower median age noted for patients from Africa. The clinical profile is essentially similar to that described for myeloma elsewhere in the world, except that the majority of our patients present with advanced stage disease (stage III). In general, the symptoms and signs are overt and exaggerated when compared to the Western world and occur in a similarly high proportion of patients as documented in other African series. The laboratory characteristics are also typical of that described. Other differences regarding the clinical and laboratory profile which have been noted in our series are highlighted.

All the patients were treated with conventional chemotherapy. The standard chemotherapy regimen used initially in the vast majority of patients was melphalan and prednisone. For refractory disease, combination intravenous chemotherapy such as vincristine and an anthracycline together with dexamethasone was used in most patients. None of the patients were subjected to peripheral stem cell or bone marrow transplantation. Poor prognostic factors of statistical significance that were found in the study following a univariate analysis include: a beta 2 microglobulin of >6mg/l (both uncorrected and corrected for renal impairment), hypercalcaemia >2.65mmol/l, urea >8mmol/l, creatinine >180μmol/l, CRP >12mg/l, platelets <100 x 10^9/l, serum paraprotein >10g/l, intermediate and advanced stage disease (based on the Durie and

Salmon classification), urine total protein >1g/l and a white cell count of <4 x 10^9/l (especially <3.4 x 10^9/l).

The case-control study showed a significantly increased risk of myeloma in agriculture (farming), but we were unable to determine the exact nature of the factor responsible for the increased risk. A significantly higher number of cases (71.5%) compared to controls (51.5%) spent time at a farm (p=0.0009; odds ratio 2.36). More of the cases (patients) spent time as the owners or workers on a farm, compared to the controls (p=0.008). The number of cases (66 - 50.7%), who worked on a farm was significantly higher than the control group (28 - 21.5%) (p=0.00005; odds ratio 3.68). There was no significant difference between the cases and controls with regard to animal exposure (p=0.635), pesticide exposure (p=0.995) or exposure to exhaust fumes (p=0.945), although in each of these categories the exposures were more in the cases than controls.

Radiation did not feature as a risk factor in our patients. With respect to benzene, lead, asbestos, mining, fibreglass/mineral fibres, working at a building construction site, work as a caterer/cook and in the forestry industry, exposure was higher in the cases compared to controls, but the difference was not statistically significant.

Regarding non-occupational exposures, there was no association between smoking or alcohol intake and myeloma. Intake of herbal toxins, ibuprofen and laxatives was higher in the cases than the controls, but the difference was not significant (NS). Allergies were higher in the cases compared to controls (NS), as were certain childhood infections such as measles and mumps. Interestingly, a significantly higher number of controls (123 - 94.6%) were immunized (against the common childhood illnesses based on the

government immunisation schedule) compared to cases (104 - 80%) (p=0.0019). Thus, immunization may play a protective role against myeloma.

The role of viruses was not specifically examined in the case-control study. However, there were only two patients of the 130 cases and no additional cases in the background 170 patients with myeloma who were HIV seropositive. The seropositive rate appears to be lower than in an aged matched hospital population without myeloma. In the KSHV/HHV-8 study, KSHV DNA sequences were detected in 4/10 (40%) of the adherent cell cultures and 1/20 (5%) of the bone marrow aspirate samples. None of the bone marrow biopsy samples (0/9), or control bone marrow aspirate samples (0/19) were positive. Based on the small sample, a similar background seroprevalence rate compared to the detection rate, as well as the conflicting data in the literature, the exact role of KSHV/HHV-8 in the aetiopathogenesis of myeloma stills remains to be elucidated and clarified.

With respect to socioeconomic factors, there were no significant differences noted in relation to home ownership, income, type of home, educational status and occupational rank (social class) between the cases and controls. Thus, socioeconomic status does not appear to have an influence on myeloma risk in our patients.

Analysis of class I and class II human leucocyte antigens in 62 myeloma patients revealed a corresponding association of statistical significance (to that documented in the literature) only with regard to HLA B18. An additional association not documented previously was HLA B35. The relative risk for myeloma in individuals with HLA B35 is 11.82. Haplotype frequencies were also studied. Haplotypes that confer a relative higher

risk of myeloma include A30,DR8; A43,B22; A43,B35; B70,Cw3 and DR11,DQ3. Those associated with a lower risk (i.e. may be protective) are A2,B42; A30,B70.

The results of the DNA ploidy analysis revealed that 63.1% of the patients had a diploid pattern, while 36.9% of the patients demonstrated aneuploidy. Hyperdiploidy accounted for 95% of the aneuploid population, while hypodiploidy was present in only 5% of the aneuploid group. The percentage of patients with aneuploidy is very variable and appears to be generally lower in our patients. When comparing the aneuploid and diploid groups, the mean plasma cell numbers were found to be significantly higher in the aneuploid group ($p=0.0005$). Additional factors of statistical significance between the two groups were a higher paraprotein level, IgG level and abnormal cytogenetics in the aneuploid group.

Plasma cell antigen co-expression of CD38-56, CD38-33, CD38-10 and CD38-45 were specifically studied. Of the four antigens, only CD38-56 expression was higher in myelomatous plasma cells. This is consistent with the reported bone marrow myeloma cell phenotype of CD56++. When the CD38-56 expression was correlated with the prognostic factors noted in this study, only stage of disease was of statistical significance ($p=0.0013$), i.e. more patients with CD38-56 expression had advanced stage (stage III disease). Furthermore, a shorter survival was noted in the CD38-56 positive group (24 months compared to the 37 months in the negative group), although the difference was not statistically significant ($p=0.1599$).

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to the following people:

Professor B.V. Mendelow, for his excellent supervision, invaluable advice, encouragement, support and guidance throughout the study, and for his critical analysis of the completed manuscript, despite his very busy schedule.

Professor A.F. Fleming, for his support and encouragement to initiate the study on the epidemiology of myeloma, and for his valuable ideas and advice on the planning of the case-control study.

Dr F. Sitas and Dr D. Kielkowsky for their expertise and advice on aspects of the epidemiology in general and the case-control study in particular. I am indebted to them for their valuable comments and assistance with structuring of the interview questionnaire in the case-control study.

Dr D.Glencross, Ms D. Lawrie, Dr N. Alli, Dr G. Sherman and all the members of the flow cytometry team, for their kind assistance and expert technical advice with respect to the flow cytometry study.

Dr G. Stevens, Dr J. Mahlangu, Mr J.B. Patel and Ms U. Allard for their technical expertise, sound advice and assistance regarding the HHV-8 study.

Professor A.A. Wadee, Ms A. Fourie and other members of the Immunology Department for their kind assistance and expertise with respect to the HLA study.

Dr W. Stevens, Dr C. Ingram, Dr C. Gavalakis, Dr D. Brittain, Mr Y. Govender, Mrs S. Ebrahim and all the medical, technical and clerical staff of the Haematology Department, SAIMR, for assisting me in various ways and for their whole hearted co-operation throughout the study.

Dr V. Philip, Dr L. Celliers, Dr G. Cohen, Dr R. Isaacs, Dr D. Lincoln, Dr S. Donaldson, Dr M. Hale, Dr D. Blumsohn, Professor M. Tikly, Professor W. Bezwoda, Dr P. Ruff, Dr A. Weaving, Sister M. Sibisi, Mrs S. Nkosi and others members (medical and nursing staff) of the Haematology unit and the registrars and consultants in the Department of Medicine, for their assistance, advice, encouragement and support.

Dr J.S. Galpin for her expert statistical analysis and advice.

To all the patients who willingly participated in the study.

To my family members and friends for their encouragement and understanding and to anyone else who I have failed to acknowledge personally.

TABLE OF CONTENTS

	PAGE
DECLARATION	ii
DEDICATION	iii
PUBLICATIONS AND PRESENTATIONS	iv
ABSTRACT	v
ACKNOWLEDGEMENTS	xi
TABLE OF CONTENTS	xiii
LIST OF FIGURES	xviii
LIST OF TABLES	xx
1.0 INTRODUCTION	1
1.1 The epidemiology of myeloma	1
1.1.1 Descriptive Epidemiology	1
1.1.1.1 Incidence	1
1.1.1.2 Mortality	8
1.1.1.3 Survival	9
1.1.2 Radiation exposures	10
1.1.2.1 Atomic Bomb survivors	11
1.1.2.2 Radiation related occupations	12
1.1.2.3 Diagnostic and Therapeutic irradiation	14
1.1.3 Occupational and Environmental exposures	16

1.1.3.1 Agriculture 17

1.1.3.2 Metals 19

1.1.3.3 Rubber manufacturing 20

1.1.3.4 Petroleum industry and fuel combustion products 21

1.1.3.5 Benzene and other chemicals 22

1.1.3.6 Wood, Leather and Textile industries 24

1.1.3.7 Miscellaneous Environmental and Occupational exposures 24

1.1.4 Non-occupational Exposures 26

1.1.4.1 Medicines 26

1.1.4.2 Smoking 26

1.1.4.3 Alcohol 27

1.1.4.4 Food 28

1.1.5 Familial and Genetic Factors 28

1.1.5.1 Familial Myeloma 28

1.1.5.2 Genetic Marker Studies 32

1.1.5.3 Chromosomal abnormalities 35

1.1.5.4 Molecular Genetics 41

1.1.6 Pre-existing medical conditions/chronic antigenic stimulation 51

1.1.7 Socioeconomic status 71

1.1.8 Aetiological heterogeneity of M-component types 72

1.2 Immunophenotype and DNA content in Myeloma 74

1.2.1 Immunophenotypic characteristics of Plasma cells 76

1.2.1.1 Antigenic characteristics of myelomatous plasma cells 78

1.2.1.1.1 B-cell related markers 78

1.2.1.1.2 Plasma cell associated markers 80

1.2.1.1.3 Adhesion molecules and natural-killer cell associated antigens 81

1.2.1.1.4 Stem cell-related markers: CD34 and c-kit 85

1.2.1.1.5 Myeloid associated markers 85

1.2.1.1.6 Other markers 86

1.2.1.2 Are normal plasma cells phenotypically different from myelomatous plasma cells? 88

1.2.2 DNA content (ploidy) of plasma cells 90

1.3 Aims of the study 94

1.3.1 Case-contol study 94

1.3.2 HLA study 94

1.3.3 HHV-8/KSHV study 94

1.3.4 Flow cytometry study 95

2.0 PATIENTS AND METHODS 96

2.1 Patients 96

2.2 Methods 100

2.2.1 Case-control study 100

2.2.2 HLA study 101

2.2.3 KSHV/HHV-8 study 102

2.2.3.1 DNA analysis 102

2.2.4 Flow cytometry study 104

2.2.4.1 Equipment 104

2.2.4.2 Materials 104

2.2.4.3 Methods 105

2.2.4.3.1 Immunophenotyping 105

2.2.4.3.2 DNA ploidy 106

3.0 RESULTS 107

3.1 General background information 107

3.2 Case-control study 124

3.3 HLA study 145

3.4 HHV-8 study 155

3.5 Flow cytometry study 159

4.0 DISCUSSION 169

4.1 General background information 172

4.2 Case-control study 179

4.2.1 Radiation exposures 180

4.2.2 Occupational and environmental exposures 181

4.2.2.1 Agriculture 182

4.2.2.2 Metals 183

4.2.2.3 Rubber manufacturing 184

4.2.2.4 Benzene and petroleum 184

4.2.2.5 Miscellaneous occupational exposures 184

4.2.3 Non-occupational exposures 185

4.2.3.1 Medicines 185

4.2.3.2 Smoking 186

4.2.3.3 Alcohol 186

4.2.4 Pre-existing medical conditions/chronic antigenic stimulation 186

4.2.5 Socioeconomic and other influences 190

4.3 HLA study 193

4.4 HHV-8 study 196

4.5 Flow cytometry study 201

5.0 CONCLUSION 206

REFERENCES 209

APPENDIX 297

LIST OF FIGURES

Figure	Page
1.1 Age-, sex and race-specific incidence rates of multiple myeloma, SEER program, USA, 1984-88	5
1.2 Summary of progressive genetic events in multiple myeloma	50
3.1 CAT Scan of chest showing a left pleural based plasmacytoma	110
3.2 Lateral skull X-ray showing multiple punched out lytic bone lesions	111
3.3 Lateral X-ray of the thoracolumbar spine showing osteopenia and vertebral compression fractures	112
3.4 Pathological fracture of the left humerus	113
3.5 Bone marrow aspirate showing prominent plasma cell infiltration	120
3.6 Kaplan-Meier survival curve for the 170 patients with myeloma	123
3.7 Kaplan-Meier survival curve for the 130 patients with myeloma	132
3.8 Agarose gel electrophoretogram from myeloma bone marrow aspirate DNA samples	157
3.9 Agarose gel electrophoretogram from myeloma bone marrow cultured dendritic cell DNA samples	158
3.10 DNA ploidy histogram in a patient with myeloma. Note the aneuploid populations depicted in yellow	161
3.11 DNA ploidy histogram in a patient with myeloma. E represents the aneuploid (tetraploid) population	162

3.12 Flow cytometric analysis - Scattergram showing dual antigen CD38-56 co-expression depicted in light blue **165**

LIST OF TABLES

Table	Page
1.1 Cytogenetic characteristics in 155 myeloma patients	**37**
1.2 Cytogenetic characteristics in 492 myeloma patients	**38**
1.3 Course of 241 patients with Monoclonal Gammopathy of Undetermined Significance	**58**
1.4 Comparison of adhesion molecule expression between normal and malignant plasma cells	**84**
1.5 Incidence of DNA hyper- and hypodiploidy, as reported in the literature	**93**
3.1 Age at presentation	**108**
3.2 Symptoms at presentation in 170 myeloma patients	**108**
3.3 Signs at presentation in 170 myeloma patients	**109**
3.4 Performance status (PS)	**114**
3.5 Stage of disease at presentation	**115**
3.6 Protein electrophoresis and Immunoglobulin characteristics	**116**
3.7 Laboratory characteristics of 170 patients at presentation	**117**
3.8 Parameters of prognostic significance in 170 patients with myeloma at presentation	**121**
3.9 Age of cases and controls	**124**
3.10 Symptoms in 130 myeloma patients (cases) at presentation	**125**
3.11 Signs at presentation in 130 myeloma patients (cases)	**125**
3.12 Performance status (PS)	**126**

3.13 Stage of disease at presentation **126**

3.14 Protein electrophoresis and Immunoglobulin characteristics (cases) **127**

3.15 Laboratory characteristics of myeloma patients (cases) at presentation **128**

3.16 Parameters of prognostic significance at presentation **130**

3.17 Comparison of various exposures in cases and controls **133**

3.18 Present address of cases and controls **137**

3.19 Birth place (cases and controls) **138**

3.20 Area lived most (cases and controls) **139**

3.21 Tribe/Language (cases and controls) **139**

3.22 Marital status (cases and controls) **140**

3.23 Highest monthly income (nett income) **141**

3.24 Home ownership (cases and controls) **141**

3.25 Number of persons per room **141**

3.26 Type of home **142**

3.27 Educational status **142**

3.28 Occupational rank (social class) **143**

3.29 Dominant occupations in the cases and controls **143**

3.30 Clinical and radiological features of 62 patients with myeloma **146**

3.31 Laboratory characteristics of 62 patients with myeloma **147**

3.32 Antigen frequencies: Class I - A locus **149**

3.33 Antigen frequencies: Class I - B locus **150**

3.34 Antigen frequencies: Class I - C locus **151**

3.35 Antigen frequencies: Class II - DR locus **151**

3.36 Antigen frequencies: Class II - DQ locus **152**

3.37 Haplotype frequencies: Higher risk **153**

3.38 Haplotype frequencies: Lower risk **153**

3.39 Published associations **154**

3.40 Patient characteristics, bone marrow and serological findings **156**

3.41 DNA ploidy analysis - comparison of the clinical and laboratory characteristics in the diploid and aneuploid groups **159**

3.42 Summary of relevant plasma cell (CD38) antigen co-expression **163**

3.43 CD38-56 antigen co-expression in relation to prognostic factors **164**

3.44 CD38-45 antigen co-expression in relation to prognostic factors **166**

3.45 CD38-33 antigen co-expression in relation to prognostic factors **167**

3.46 CD38-10 antigen co-expression in relation to prognostic factors **168**

CHAPTER 1

1.0 INTRODUCTION

1.1 THE EPIDEMIOLOGY OF MYELOMA

Multiple myeloma (myeloma) is a malignancy of plasma cells. Although the exact aetiology of myeloma remains obscure and its cause unknown, a number of risk factors have been implicated in its pathogenesis. Among these are genetic, occupational, environmental and socioeconomic influences that have been linked to, or may interact in the causation of the disease.

In this chapter, the role of the above mentioned risk factors will be examined.

1.1.1 Descriptive Epidemiology

1.1.1.1 Incidence

The reported increases in the incidence of myeloma a few decades ago (McPhedran et al, 1972; Kyle et al, 1969; MacMahon and Clark, 1956), especially in the elderly, engendered much interest in the epidemiology of the disease. Much of the concern centred on the question of whether the trends reflect a true increase in the incidence of the disease or whether they are due to improved case ascertainment. Reports in the literature on the incidence and time trends for the malignancy suggest that the overall incidence is now relatively stable (Vineas et al, 1996; Cuzick 1994; Hansen et al, 1989; Velez et al, 1982), and that the prior increases noted were probably related to increased availability and utilization of medical facilities and improved diagnostic techniques, particularly in the older population (see later).

The incidence of myeloma varies substantially. Patients have been reported from all parts of the world, and no race is known to be immune. On average, the incidence is approximately 4/100 000 per year (Kyle et al, 1994).

Incidence data in the United States of America (USA), as reported by the Surveillance, Epidemiology and End Results (SEER) program, for the period 1984 to 1988, indicate that myeloma accounts for 2% of all malignancies in blacks and 1% of all malignancies in whites (Ries et al, 1991). Myeloma is the most common lymphoid malignancy in blacks, while non-Hodgkin's lymphoma ranks first among whites in the USA (Devesa et al, 1987). Myeloma accounts for 31% of the lymphohaematopoietic malignancies among blacks and 13% among whites (Ries et al, 1991).

The highest annual age standardized (age 35-64 years, 1978-1982) incidence rates have been reported for African-Americans (9-13,9/100 000 males, 3,9-9,8/100 000 females) and native Pacific Islanders (e.g. Pacific Polynesian Islands 20,5/100 000 males, 5,7/100 000 females; Maori 7/100 000 males, 6,8/100 000 females; Hawaiian 6/100 000 males, 6,4/100 000 females). Intermediate rates have been reported from Europeans and North American Caucasians (e.g. Norway 5,1/100 000 males, 2,9/100 000 females; Switzerland 3,2-5,7/100 000 males, 1,4-4,8/100 000 females), while generally low rates have been reported for Asians living in Asia and the USA (e.g. China 0,3-1,1/100 000 males, 0,6/100 000 females; Japan 1,2-2,1/100 000 males, 1,1-1,6/100 000 females) (Muir et al, 1987). Further data, reported more recently by Parkin et al (1992), (incidence rates per 100 000/year, age adjusted on the world population, 1983-1987), show similarly high rates in myeloma for African-Americans in Detroit (9/100 000 males) and Alameda (6,6/

100 000 females), while the lowest rates were reported from Algeria (0,2/100 000 males) and Kyrgyzstan and Peru (both 0,1/100 000 females). It is apparent from these data that there are both striking geographic as well as ethnic differences in myeloma incidence. This brings into question the role of environmental and genetic factors in the causation of myeloma. Viruses, with particular reference to the human herpes-virus 8 (HHV-8), may be implicated as a potential environmental agent (see later). The low incidence of myeloma in the Chinese and Japanese has moved with them to Hawaii and the USA (Devesa, 1991), suggesting that the incidence of the disease is also influenced by genetic factors (in this instance, more than by environmental factors).

Comparison of incidence rates from international cancer registries must be made cautiously, since the populations served may vary in size and age distribution and may vary with regard to accuracy of case ascertainment (e.g. pathology versus death certificate only), level of medical care and diagnostic accuracy (Muir et al, 1987; Parkin, 1986; Waterhouse et al, 1982).

In South Africa, the age standardized incidence rates for the whole population, based on data from the National Cancer Registry, for 1990 and 1991 is 1,79/100 000 males and 1,33/100 000 females (Sitas et al, 1996). A slight increase has been noted compared to the rates for 1987 (1,5/100 000 males, 1,0/100 000 females) (Cancer Registry of South Africa, 1987). This may in part be explained by improved case ascertainment (greater access to medical facilities, earlier diagnosis, better reporting), with particular reference to blacks, as the incidence rates have increased for black males and females (1987 - 1,3/100 000 males, 1,0/100 000 females; 1990 & 1991 - 1,86/100 000 males, 1,44/100

000 females), compared to a decrease for both white males and females (1987 - 2,3/100 000 males, 1,1/100 000 females; 1990 & 1991 - 1,40/100 000 males, 0,95/100 000 females) (Sitas et al, 1996; Cancer Registry of South Africa, 1987). Based on the 1990 & 1991 incidence data, the incidence rates are higher in blacks than whites. Although these data do not appear striking, with continued improvements in the socioeconomic status of the population (majority blacks), it is possible that the differences in the incidence may become more marked in these two ethnic groups. Further support of the high rate of myeloma in South African blacks is based on the report by Blattner et al (1979), who surveyed myeloma cases in Johannesburg between 1973-1975, and found that the age adjusted (world standard) rates for black males (7,47/100 000) and females (5,1/100 000), closely resembled the incidence rates reported for blacks in the USA. The results of this study suggest that the high rate for myeloma in blacks is not limited to the USA. The incidence rates quoted in this study appear to be at variance with the figures quoted earlier from the National Cancer Registry. Some of the reasons for these differences will be mentioned. The National Cancer Registry data provides information for the whole population (including the rural and urban areas), while the study of Blattner et al (1979), was conducted in Johannesburg (at two academic hospitals), where there is greater awareness of the disease and specialised diagnostic tests are more readily available. Moreover, in general, there is underreporting of myeloma cases to the National Cancer Registry, as the reporting in the past was traditionally based only on histological evidence of the disease. This erroneous method of reporting myeloma cases has subsequently been corrected (at least at the Chris Hani Baragwanath Hospital).

Age, sex and race-specific incidence data (1984-1988) from the SEER program, USA, are shown in Figure 1.1. Based on these data, the incidence of myeloma in the USA increased with increasing age, males had higher incidence rates than females and African-Americans had higher rates than caucasians (Ries et al, 1991).

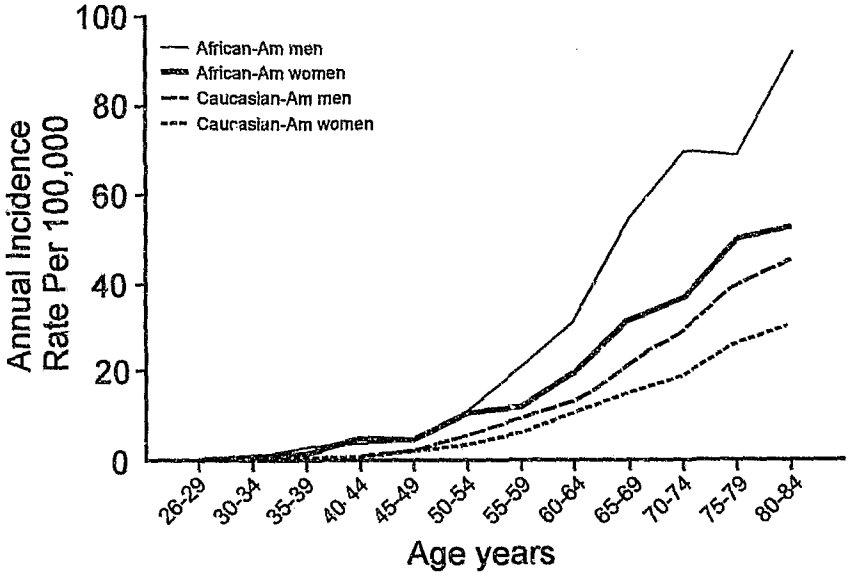


Figure 1.1 Age-, sex, and race-specific incidence rates of multiple myeloma, SEER program, USA, 1984-88. (From data reported by Ries et al, 1991).

Myeloma is characteristically a disease of middle and old age. The incidence of the disease increases with age and reaches a peak during the seventh decade of life. The median age at diagnosis is approximately 65 years. In the USA, the median age at diagnosis for myeloma is slightly higher (69 for men and 71 for women), compared to the median age of diagnosis for all cancers (68 for men and 66 for women respectively) (Ries et al, 1991). In the Western world, fewer than 2% of the patients are less than 40 years of age at diagnosis (Hewell and Alexanian, 1976). Myeloma case series from Africa have suggested a younger median age at diagnosis (approximately ten years younger) than in the Western world, probably reflecting the younger age structure of the population (Patel et al, 1992; Jacobson, 1991; Mukiibi and Kyobe, 1988). These studies also report a male excess, which is consistent with the gender difference (male predominance) reported in other countries (Cuzick et al, 1983).

In a review by Herrinton et al (1995), the secular changes in myeloma incidence were examined. Three studies were conducted in areas where a high level of case ascertainment were expected, viz., i) Olmsted County, Minnesota, USA; ii) Malmö, Sweden; and iii) Canton of Vaud, Switzerland. In Olmsted County (where the Mayo Clinic is located), the annual age-standardized incidence of myeloma remained constant from 1945-1977 at approximately 3,5/100 000 males and 2,2/100 000 females (1950 US standard) (Linos et al, 1981). In Malmö, where the medical community have had a close interest in myeloma since the 1960s, the incidence rate in men increased by 60% between 1950 and 1979 to an annual rate of 4,6/100 000 (1950 US standard) among all age groups, while no increase was observed in the rate among women (average annual

incidence was 2,7/100 000) (Turesson et al, 1984). In the Canton of Vaud, no changes in incidence were noted between 1978 and 1987 (average annual incidence rates were 4,8/100 000 in males and 2,7/100 000 in females) (European standard) (Levi and La Vecchia,1990).

Devesa et al (1987), studied the secular changes in incidence among residents of four geographic areas of the USA (Atlanta, Connecticut, Detroit and San Francisco/Oakland) for the period 1947-1984. Between 1947 and 1975, the annual incidence rates increased by about 150% to 3,8/100 000 in males and 2,6/100 000 in females (1950 US standard). No increases were noted between 1975 and 1984. The age-adjusted incidence of myeloma in African-Americans did not change appreciably from 1973-1988 (Ries et al, 1991). Other studies with regard to changes in incidence rates over time have also been documented. In Denmark, the annual incidence increased between 1943 and 1962 from 1,3 to 3,3/100 000 males and from 1,2 to 2,5/100 000 females (European standard). No increase was observed between 1963 and 1982 (Hansen et al, 1989). In Connecticut, a 10-fold increase in incidence in men and women was observed between 1935 and 1975 (Zheng et al, 1992). In Western Australia, Israel and New Zealand, the annual incidence increased throughout the 1970s (Nandakumar et al, 1988; Shapira and Carter, 1986; Pearce et al, 1985).

An analysis of these reported studies by Herrinton et al (1995), suggests that the increase in the incidence of myeloma was predominantly the result of changes in case ascertainment. This is based on the following evidence, viz., i) there was no increase in the incidence of myeloma in the Canton of Vaud or in Olmsted County, both are areas

with good surveillance for the disease, ii) in the countries with relatively high incidence, the rates stabilized since the 1970s and iii) increases in incidence were highest among older individuals (a group in whom a laboratory diagnosis might less commonly have been sought in the past than presently). Thus, it appears that changes in the availability and use of serum protein electrophoresis, immunoelectrophoresis and immunofixation - all relatively sensitive diagnostic methods were likely to account for the reported increases in incidence in some locations. This, however, does not explain the increase of myeloma in men compared to women. In this instance, the introduction of an occupational agent, with particular reference to the population in Malmö, Sweden, may serve as a better explanation (Turesson et al, 1984).

1.1.1.2 Mortality

Myeloma is responsible for about 1% of all cancer-related deaths in Western countries (Riedel and Pottern, 1992). Myeloma mortality has reportedly increased in a number of countries (Cuzick, 1990; Kato et al, 1985; Cuzick et al, 1983; Ludwig et al, 1982; Velez et al, 1982; Blattner et al, 1981). The increase is most marked in individuals over the age of 55 (Cuzick, 1994; Davis et al, 1990). In general, countries with low death rates, such as Japan, showed the largest increase in mortality. Countries with the highest death rates, such as Norway, had the lowest increase. Intermediate to relatively high rates were noted in the United Kingdom (Cuzick, 1994). In a recent review by Parker et al (1997), the mortality rates in the USA (per 100 000 for 1988-1992) for males and females are as follows, viz., African-American males 7,3/100 000, white males 3,4/100 000 and

African-American females 5,0/100 000, white females 2,2/100 000 (Parker et al, 1997). The mortality pattern closely parallels the incidence curves; the median age at death is 70 years for men and 71 for women (Blattner et al, 1980a). A similar pattern of increase is noted among men and women (Parker et al, 1997; Cuzick, 1990). From an epidemiological point of view, this suggests that a common environmental factor rather than occupational exposure may have more influence on mortality (Cuzick, 1990).

1.1.1.3 Survival

The median survival of patients with myeloma prior to the introduction of effective alkylating agent and systemic combination chemotherapy was less than 1 year (Holland et al, 1966; Korst et al, 1964). With conventional chemotherapy, the median survival is now in the range of approximately 3 years (Bergsagel, 1995). The situation may improve further in patients who are diagnosed earlier and who respond to treatment (especially younger individuals who may benefit from bone marrow transplantation) and those who receive high dose chemotherapy followed by peripheral blood stem cell transplantation (Bladé, 1998).

Trends of survival in the USA show the following 5 year survival rates (%), viz., 1960-1963 : 12%; 1970-1973 : 19%; 1974-1976 : 24%; 1980-1982 : 28%; 1986-1992 : 28% (Parker et al, 1997).

Conflicting reports appear on the survival of myeloma patients, based on the influence of epidemiological factors. With regard to SEER cases diagnosed from 1981 to 1987, blacks in the USA had a slightly more favourable 5 year relative survival rate than their

white counterparts, i.e. 28,3% and 26,3% respectively. (Ries et al, 1991). This is in contrast to a study conducted by Savage et al (1983), which revealed poor survival rates in blacks with myeloma. Several indices of poverty best explained the low survival pattern. Even when cases were matched for clinical stage and race, the indices of poverty were the best correlates of poor survival. These individuals have less access to medical facilities or seek medical attention at a more advanced stage of their disease, with resultant poorer survival rates. In addition, the SEER data showed that the relative survival rates were higher for women than for men, irrespective of race: 29,3% versus 27,5% for blacks and 27,5% versus 25% for whites (Ries et al, 1991).

A number of studies have showed that survival is generally better among those with higher income and education (Glover et al, 1991, Pasqualetti et al, 1990, Savage et al, 1983). However, other studies do not support this viewpoint (Lenhard et al, 1987; Weston et al, 1987). Lenhard et al (1987), showed in their study of USA myeloma patients that survival was related to the patient's ability to travel to receive treatment, perhaps reflecting better prognosis.

1.1.2 Radiation Exposures

One of the more convincing risk factors for myeloma has been exposure to ionizing radiation. This has been documented in the studies of survivors of the atomic bombs in Hiroshima and Nagasaki, Japan, in 1945 (Ichimaru et al, 1982). Radiation exposures include exposure to the atomic bomb, radiation related occupations such as nuclear workers and radiologists, and recipients of therapeutic and diagnostic radiation.

In contrast to individuals who were exposed to high dose irradiation , the excess risk of myeloma in association with low dose radiation exposure remains controversial. Ionizing radiation is potentially carcinogenic. The target of ionizing radiation is believed to be DNA, or more precisely the proto-oncogenes in DNA.

1.1.2.1 Atomic Bomb Survivors

The strongest association between radiation and myeloma has been in atomic bomb survivors (Shimizu et al, 1990; Hayakawa et al, 1989). Individuals who entered the bombed areas in the city of Hiroshima within 3 days after the blast had nearly 60% greater risk of myeloma mortality than those not exposed (Hayakawa et al, 1989). Among the atomic bomb survivors in Hiroshima and Nagasaki, 36 deaths occurred from myeloma during 1950-1985. Using DS-86 (Dosimetry System-86) revised doses, it was estimated that there was a 3,3 fold increase in mortality (RR=3,3) per Gray (Gy) of radiation delivered to the bone marrow (95% CI, 1,7-6,3) (Shimizu et al, 1990). (1 Gy=100 rad).

More than 99% of the radiation from the atomic bomb was reported as gamma radiation; the dose in Sievert (Sv) would be approximately equal to the dose in Gy in this study. The ratio of external dose to bone marrow dose was 1,25:1.

Ichimaru et al (1982), examined risk in relation to dose and time since exposure and found that among 109 000 survivors, 29 myeloma deaths occurred during 1950-1976, and the risk of death increased with time since exposure and increased dose. He concluded that there was a statistically significant increased incidence of myeloma among

the survivors exposed to radiation dose estimates of more than 1 Gy, after the atomic bombs at Hiroshima and Nagasaki, although close scrutiny of these results do not appear to support the increased incidence suggested.

In general, myeloma occurs after a long latent period (approximately 20 years), in atomic bomb survivors exposed to high doses of radiation (Shimizu et al, 1990). The Life Span Study of Atomic Bomb Survivors by the Radiation Effects Research Foundation has added 12 years of follow-up for the occurrence of myeloma. Interestingly, a re-analysis of the data, using DS-86 dose estimates, with re-evaluation of all the cases included in previous studies and an estimate of the excess absolute risk (EAR), has changed the conclusions about the effect of radiation on the incidence of myeloma (Preston et al, 1994). This study showed that the risk (EAR) of myeloma was not increased.

Also, the frequency of monoclonal gammopathy in survivors of the atomic bombs does not appear to be increased, as measured by cellulose acetate electrophoresis (Neriishi et al, 1993).

1.1.2.2 Radiation Related Occupations

Radiation related occupations include radiologists, radium dial painters and the nuclear industry. The excess of myeloma deaths among American radiologists (1948-1961) is well documented (Lewis, 1963). He stated that there was a 5-fold increased incidence of dying from myeloma (95%CI, 1,6-12). A more recent study showed that radiologists exposed to lower doses of long-term radiation had a twofold excess myeloma risk (Matanoski, 1982). However, in China, no myeloma cases were diagnosed in radiology

workers, although only 0,5 cases were expected among the 27 000 radiology workers (Wang et al, 1988).

Women who were employed in the US radium dial painting industry have been reported to have an increased incidence and mortality rate from myeloma (Stebbings et al, 1984). Large exposures to radium resulted from licking bushes dipped into radium-containing paint, to make fine tips. Accurate measures of exposure were lacking. It appears that the higher incidence and mortality were more strongly associated with length of employment rather than with internal radium intake.

An increased risk of myeloma mortality has been observed among workers in the nuclear industry (Gilbert et al, 1989b; Smith and Douglas, 1986; Mancuso et al, 1977; Dolphin, 1976). Gilbert et al (1989a), showed in a combined analysis of mortality data from the Hanford site, Oak Ridge National laboratory and Rocky Flats Nuclear Weapons Plant that myeloma was the only cancer with a significantly increased risk. In most instances, chronic exposure at the workplace for 10-15 years were necessary for the development of myeloma (Gilbert et al, 1989b; Smith and Douglas, 1986). At the Sellafield nuclear fuels plant, United Kingdom (UK), a direct relationship between dose and mortality was observed, with workers receiving an external dose of >0,200 Sv being at 5-fold increased risk, relative to the general population (Smith and Douglas, 1986), while in another study persons exposed occupationally to considerably lower levels of external radiation (<0,01 Sv) showed no excess risk of myeloma (Beral et al, 1985).

The American Cancer Society case-control study noted a 1,9 fold increase in mortality (95%CI, 0,8-4,8) following occupational exposure to X-rays and radioactive materials (Boffetta et al, 1989).

Interestingly, residential proximity to nuclear facilities have provided little or no evidence of an increased risk of myeloma (Jablon et al, 1990; Cook-Mozzaffari et al, 1989; Dousset, 1989; Forman et al, 1987).

Two studies of workers who participated in atmospheric nuclear weapons testing have been reported. The initial report in British workers suggested an increased risk of death from myeloma compared to controls (Darby et al, 1988), but a subsequent follow up study showed that neither the incidence nor mortality from the disease is exceptionally high in participants compared to controls (Darby et al, 1993). The authors suggest that the excess of myeloma in the participants compared with controls reported previously seems likely to have been a chance finding. New Zealand participants in the same nuclear weapons tests were not at an increased risk (Pearce et al, 1990).

1.1.2.3 Diagnostic and Therapeutic Irradiation

The radiation doses received during both therapeutic and diagnostic irradiation are directed at a focal area, whereas the doses received by occupational exposure and atomic bomb survivors, tend to be relatively uniform over the body. Moreover, the dose received during therapeutic irradiation can be high.

Associations between myeloma and low dose radiation exposure (e.g. diagnostic X-rays) are controversial. No increase in the risk of myeloma following diagnostic X-ray

exposure has been noted in a number of studies (Boffetta et al, 1989; Davis et al, 1989; Cuzick and De Stavola, 1988). This varies from simple diagnostic X-ray exposure (Davis et al, 1989), to individuals receiving nine or more X-rays in a UK based case-control study (Cuzick and De Stavola 1988). However, in a Swedish case-control study conducted between 1973 and 1983, individuals who received 'heavy' levels of X-ray examinations were at 2,9 fold increased risk of myeloma (95%CI, 0,4-19) compared to those who received 'light' levels (Flodin et al, 1987). A further study by Eriksson (1993), also confirmed that significant exposure (21 or more examinations) is associated with increased risk (OR= 0,9, 95%CI, 0,4-2,1) compared to lesser exposure (6-10 examinations) (OR= 0,5, 95% CI, 0,3-0,9). At Kaiser Permanente, northern California, bone marrow doses of 0,04 Gy or greater, resulted in an increased myeloma risk of 3,9 fold (Boice et al, 1991).

Although there is evidence that increasing doses were related to increasing risk, categorising data on the basis of number of procedures may result in misclassification. Boice et al (1991), have noted that five or fewer procedures resulted in a dose to the bone marrow in the range of 0,00001-0,03 Gy, while 15 or more X-ray procedures had a range of 0,001-0,23 Gy. The discordance between the number of prior X-ray examinations and bone marrow dose was caused by differences in procedures, for example, an upper gastrointestinal procedure contributed 60 times more radiation to the bone marrow than a chest X-ray.

Regarding therapeutic irradiation, reports of an increased risk of myeloma have been inconsistent. Boice et al (1985), showed that the risk of myeloma was 2-fold increased

among women (95%CI, 1,1-3,2), who were estimated to have received a mean marrow dose of 10 Gy during therapy for cervical cancer, after 15 years.

In another case-control study by Boice et al (1988), no excess risk was noted among women who received an average marrow dose of 7,1 Gy compared to those who received less than 2,0 Gy (OR= 0,3; 90% CI, 0,1-0,4).

Furthermore, a study of patients in England and Northern Ireland receiving therapeutic radiation for ankylosing spondylitis showed a modest elevation in myeloma risk (RR=1,7) (Darby et al, 1987). A subsequent study looking at cancer mortality following X-ray treatment for ankylosing spondylitis showed a greater than expected mortality for a number of cancers, including myeloma (Weiss et al, 1994).

1.1.3 Occupational and Environmental Exposures

The role of occupational exposures as a risk factor in myeloma remains uncertain. Myeloma is not a common malignancy, and, as such, cohort studies of the relationship between occupational exposures and the disease have provided mostly, only limited information. In cohort studies where census data were used, job title as recorded in the census was the only occupational exposure variable that could be evaluated. Some of the case-control studies are of limited power as well, in that only a few subjects had worked in the occupations of interest and only abbreviated histories could be obtained. Two notable exceptions, among others, are the studies by Heineman et al (1992a) and Pottern et al (1992a). Another problem is that the specific chemical or physical agent may be known to the respondents by different names. Also, it is possible that agents known to be

potentially hazardous are better recalled by cases than controls. A further difficulty in analysing the studies, concerns the varied occupational practices and standards in use over time and place. Additionally, in many of the occupations, exposure to a wide variety of agents occurs, making causal associations to specific chemical and physical agents less clear. Lastly, data on environmental and occupational factors must be interpreted cautiously, because some associations may have occurred by chance alone.

Despite these problems, there are a number of reports in the literature which suggest a possible association between occupational exposure and myeloma. Most of these are in relation to agriculture (farming) (Demers et al, 1993; Riedl et al, 1991; Cuzick and De Stavola, 1988), but the list includes exposure to metals, asbestos, benzene and petroleum, paints and solvents, rubber, wood, leather, food processing and a host of miscellaneous occupational exposures (see below).

1.1.3.1 Agriculture

A significantly increased risk for myeloma in agriculture (particularly farming) has been reported in a number of studies (Demers et al, 1993; Riedl and Pottern, 1992; Blair and Zahm, 1990; Boffetta et al, 1989; Brownson et al, 1989; Cuzick and De Stavola, 1988; Nandakumar et al, 1986; Wiklund and Holm, 1986; Burmeister et al, 1983). Fewer studies have shown no association (Zahm et al, 1992; Burmeister, 1990; Reif [illegible] 1989; Brownson and Reif, 1988; Tollerud et al, 1985). Milham (1971), first reported significantly increased mortality in farmers from myeloma, in a study based on

Washington state deaths from 1950-1971. A similar report in Wisconian farmers was published by Cantor and Blair (1984).

In the reports in which an association has been suggested, because of the wide variety of different exposures, it is not possible to determine whether oncogenic zoonotic viruses, pesticides (including herbicides and insecticides), agricultural chemicals or some combination of these exposures is responsible for the increased risk of myeloma. A few studies considered duration of employment as a farmer. A trend of increasing relative risk was noted in three studies (Demers et al, 1993; Boffetta et al, 1989; Alavanja et al, 1988), while the fourth did not show an increased risk (Heineman et al, 1992a).

Other associations or specific exposures that may increase myeloma risk among farmers include dairy and sheep farming (including zoonotic infections), exposure to cattle, poultry and other farm animals, orchard farming, exposure to grain dust, aflatoxins, paints and solvents, wood treatment, chemicals used for fencing, engine exhaust from farm equipment, welding fumes and pollen (Pearce and Reif, 1990; Blair et al,1985).

In an Iowa study, Burmeister et al (1983), reported that one of the agricultural practices most consistently associated with mortality from myeloma was the raising of egg-laying chickens. A potential link was cited with Marek's disease, a herpes virus-induced malignant lymphoma in chickens, with reticuloendothelial neoplasms in humans. In a recent review of the cancer incidence among Finnish farmers (1979-1993), an excess of myeloma cases was found among farmers on pig or poultry farms, but not on other types of farms (Pukkala and Notkola, 1997).

Regarding agricultural pesticides, Morris et al (1986), showed a 2,5 fold increased risk of myeloma. Boffetta et al (1989), found that the risk increased significantly for those exposed to both farming and pesticides (pesticides alone, OR=1; farming alone, OR=1,7; both pesticides and farming, OR=4,3), whereas the risk was not increased appreciably among those who were exposed only to farming or only to pesticides. Demers et al (1993), showed a similar increased odds ratio (OR=7,9), for exposure to both pesticides and farming. In neither of the two studies did pesticide use appear to explain completely the relationship between agricultural work and myeloma. Three further studies showed no significant association between myeloma and pesticide use (Brown et al, 1993; La Vecchia et al, 1989; Wiklund et al, 1989).

Herbicides potentially associated with myeloma include phenoxyacid herbicides and chlorophenols (Blair and Zahm, 1990; Pearce et al, 1986), DDT and mercurial seed dressing (Eriksson and Karlsson, 1992). Burmeister (1990), found an elevated myeloma risk for exposure to the crop insecticides of the organochlorine class, and for the dinitroaniline, thiocarbamate and urea classes of herbicides.

1.1.3.2 Metals

Exposure to various metals have been linked with myeloma. A significantly increased risk has been observed among sheet metal workers and smelter and metallurgy workers (McLaughlin et al, 1988), while non-significant associations have been reported for foundry workers (Giles et al, 1984; Spinelli et al, 1984). Morris et al (1986), in a case-control study reported an association between myeloma and arsenic, cadmium, copper

powder and fumes, and lead vapours and liquid. These excesses occurred among whites but not among blacks.

At a Canadian nickel refinery, exposed workers had a significant increase in myeloma mortality (Egedahl et al, 1991), while in contrast, no increase in myeloma/lymphoma mortality was noted among Swedish workers grinding stainless steel, who were potentially exposed to cadmium and nickel (Svensson et al, 1989).

Trace levels of lead and cadmium in US water supplies has been associated with myeloma mortality (Berg and Burbank, 1972). However, Linet et al (1987), did not find lead exposure to be a risk factor for myeloma. A cohort study of chimney sweeps reported a marginally significant increase in myeloma incidence. Potential occupational exposures included polycyclic aromatic hydrocarbons (formed by combustion of coal, wood, coke and oil) and metals such as arsenic, nickel and chromium (Gustavsson et al, 1988).

1.1.3.3 Rubber Manufacturing

Rubber manufacturing may be incriminated as a risk factor for myeloma (Cuzick and De Stavola, 1988; Gustavsson et al, 1986). Rubber workers can be exposed to organic solvents, plastic monomers, rubber additives and asbestos among other agents, and in the past, exposure to benzene was high. Increased mortality rates have been observed in rubber workers in the USA, where rubber bands, belts, hoses and molded rubber goods were made (Andjelkovich et al, 1978; Monson and Nakano, 1976). In the Swedish study (Gustavsson et al, 1986), the slight increase in myeloma risk was associated with longer

exposure time to the chemical weighing and mixing process. A further study in the British industry, however, did not show any link with myeloma (Sorahan et al, 1989).

1.1.3.4 Petroleum Industry and Fuel Combustion Products

Some of the known carcinogens to which petroleum workers are exposed include polycyclic aromatic hydrocarbons and various solvents, which in the past may have included benzene. An excess mortality rate due to cancer of the lymphatic system has been observed for exposures to petrochemical products (Marsh et al, 1991; Wong and Raabe, 1989; Linet et al, 1987; Kaplan, 1986; Divine et al, 1985). Linet et al (1987), showed that the observed rate of myeloma for a history of occupational exposure was 3,7 times the expected rate. The increased rate of myeloma in petroleum workers correlates well with the mineral oil-induced model for plasma cell neoplasms in BALB/c mice (Metcalf, 1974). Other studies have not showed an excess mortality rate (Divine and Barron, 1987; Hanis et al, 1985), including a study of highway maintenance workers potentially exposed to asphalts, tars, gasoline, engine exhaust and lead (Bender et al, 1989). In contrast to these negative reports, exposures to carbon monoxide as a consequence of exposure to diesel, jet fuel, or automobile exhausts, coal fumes and smoke have all been linked with myeloma risk (Cuzick and De Stavola, 1988; Flodin et al, 1987; Morris et al, 1986). There is now a large body of information on the carcinogenic hazards of firefighting. Myeloma is included in the list of cancers prevalent among firefighters (Golden et al, 1995).

1.1.3.5 Benzene and other chemicals

The metabolites of benzene are known to be potentially marrow toxic (Snyder et al, 1993; Decouflé et al, 1983). Benzene is a versatile industrial chemical. It is a natural component of crude and refined petroleum products. It is also formed in the combustion of organic materials. Benzene is used primarily as a raw material in the manufacture of synthetic organic chemicals. In the past, benzene was used extensively as an organic solvent. It formed an important component of paint thinners, adhesives, and degreasing compounds. It is also present in many organic compounds as a contaminant. Benzene exposure has been associated with myeloma (Goldstein, 1990; Aksoy et al, 1984; Torres et al, 1970). In another study, an excess of myeloma and leukaemia mortality was observed among workers exposed to benzene in the manufacture of rubber hydrochloride in pliofilm plants, by a process which includes the dissolution of natural rubber in benzene (Rinsky et al, 1987). An update of this study has added seven years of follow-up (Paxton et al, 1994). No new cases of myeloma have developed, and the standardized mortality ratio for myeloma is no longer elevated significantly in this benzene-exposed cohort. In a case-control study of chemical workers, there was an association with benzene exposure of five years or more (Ott et al, 1989), while in another case-control study no association was reported (Linet et al, 1987). In an update entitled, "Does benzene cause multiple myeloma?", Bezabeth et al (1996), reviewed the population-based and hospital-based case-control studies published through mid-1995 regarding the relationship between myeloma and benzene exposure or surrogates for benzene exposure. No increased association was found between myeloma and benzene exposure

or exposure to chemical groups that include benzene. The odds ratio from these analyses approximated 1,0. Exposures to petroleum products and employment in petroleum-related occupations did not appear to be risk factors for myeloma. Another review also does not indicate a positive association between myeloma and benzene exposure (Savitz and Andrews, 1997).

Furthermore, data from cohort studies of petroleum workers (the majority being refinery workers) were reviewed and pooled by Wong and Raabe (1997). The pooled analysis indicates that petroleum workers are not at an increased risk of myeloma as a result of their exposure to benzene, benzene containing liquids, or other petroleum products in their work environment.

Bergsagel et al (1999), in their recent review of benzene and myeloma conclude that in contrast to the strong evidence linking high levels of benzene exposure to an increased risk of developing acute myelogenous leukemia, there is no scientific evidence to support a causal relationship between exposure to benzene or other petroleum products and the risk of developing myeloma.

A number of other chemicals have been associated with an excess myeloma mortality. These include piperazine, urethane, formaldelyde, methylene chloride, benzyl chloride, ethylene oxide, perchlorethylene and epichlorohydrin (Spirtas et al, 1991; Hayes et al, 1990; Ott et al, 1989; Hagmar et al, 1986).

In a Canadian study, a significant increase of myeloma was observed among machinists, who were potentially exposed to hard metallic alloys, cutting oils and mineral oil lubricants in the production of machine parts (Gallagher and Threlfall, 1983).

1.1.3.6 Wood, Leather and Textile Industries

In the wood industry an association with myeloma has been mostly with the pulp and paper making processes (Schwartz, 1988; Flodin et al, 1987).

With regard to the leather industry, workers in the leather tanning industry had a nearly 2 fold, but non-significant increased risk of myeloma (Cuzick and De Stavola, 1988). In a study involving shoe manufacturing workers a 2 fold increase was noted among men and >3 fold increase in women (Walrath et al, 1987).

Among women textile workers employed in the wool industry in Sweden, a 3 fold increase risk of myeloma was noted, but two other studies in textile workers failed to confirm a similar increased risk (Dubrow and Gute, 1988; Delzell and Grufferman, 1983).

1.1.3.7 Miscellaneous Environmental and Occupational Exposures

There are reports of positive associations between myeloma and hair dye application and usage (Spinelli et al, 1984; Guidotti et al, 1982). Another study showed a modest association of regular use of hair dyes (OR=1,5;95%CI,0,75-2,9), with myeloma among men but not among women (Herrinton et al, 1994). A further study by La Vecchia and Tavani (1995), was inconclusive, primarily because of the small number of exposed cases, thus hampering the interpretation of results. Hair dyes (colouring agents) are known to contain constituents, including aromatic, nitro, and amino compounds that are carcinogenic or mutagenic in laboratory animals (Ames et al, 1975).

Painters are exposed to dyes and pigments, dusts, aromatic and aliphatic hydrocarbons and low-molecular-weight solvents such as trichloroethylene and methylethylketone (Bethwaite et al, 1990). Exposure to paint and myeloma risk have been reported in case-control and prospective studies (Demers et al, 1993; Bethwaite et al, 1990; Cuzick and De Stavola 1988; Lundberg 1986; Morris et al, 1986).

In New Zealand, a higher myeloma risk was observed among car spray and sign painters than among general painters (Bethwaite et al, 1990). Higher risk for spray painters may be due to increased exposure to non volatile components in the paints.

An association between asbestos exposure and myeloma has also been reported in a number of studies (Raffn et al, 1989; Cuzick and De Stavola, 1988; Linet et al, 1987). This was more significant in individuals who were exposed for at least 10 years or more (Cuzick and De Stavola, 1988). Linet et al (1987), showed an observed rate of 3,5 times the expected rate of myeloma following exposure to asbestos.

An interesting observation has been made with regard to myeloma and dioxins (Schwartz, 1997). Clusters of myeloma have occurred in close proximity to bodies of water contaminated by dioxins. Exposure to dioxins occur in individuals who consume local fish and seafood. This hypothesis is consistent with the significantly elevated risks for myeloma in groups with high consumption of dioxin-contaminated fish, e.g. Baltic Sea fisherman. Dioxins are immunotoxic and inhibit differentiation of B cells. Thus dioxins may be potential myelomagens (Schwartz, 1997).

1.1.4 Nonoccupational Exposures

Nonoccupational exposures include the use of prescription and over-the-counter medicines, smoking, alcohol and food intake. Medications have been suggested as myeloma risk factors in several case-control studies, while intake of various foods, smoking and alcohol consumption have generally not been found to be significantly associated with myeloma.

1.4.1 Medicines

Although a number of medications have been associated with increased myeloma risk, it is unclear whether the drug or the underlying medical condition being treated is the responsible factor. The probability of a chance finding can also not be ruled out.

Drugs that have been incriminated include propoxyphene (Friedman, 1986), phenytoin, phenobarbitone, propranolol, diazepam, ibuprofen, diet drugs, stimulants, laxatives (Linet et al, 1987), erythromycin, chlorpheneramine, gentamycin sulphate, sulfamethoxazole and terpin hydrate (Selby et al, 1989).

1.1.4.2 Smoking

Most studies looking at smoking as a risk factor have shown no relationship between tobacco use and myeloma occurrence (Friedman, 1993; Brown et al, 1992; Heineman et al, 1992b; Herrinton et al, 1992; Linet et al, 1992; Brownson, 1991; Gramenzi et al, 1991; Boffetta et al, 1989; Linet et al, 1987; Gallagher et al, 1983; Williams and Horm 1977). However, in a Swedish case-control study, ex-smokers had an elevated risk, but

current smokers did not (Flodin et al, 1987). A prospective cohort study of 34 000 U.S. S. . th Day Adventists followed for six years detected nine cases of myeloma in ex-smokers, and two in current smokers. This s' dy showed an association for number of cigarettes smoked in current smokers (OR=6,8, 95%CI, 1,4-33,6) and past smokers (OR=3,0, 95%CI 1,1-8,1), with a trend of increasing risk with increasing number of cigarettes smoked per day, e.g. 1-14 cigarettes per day (OR=1,4, 95%CI 0,3-6,8), versus 25 or > cigarettes per day (OR=4,7, 95%CI 1,3-17,3), but not with number of years smoked (Mills et al, 1990). This group will have to be followed up longer because this positive result differs from the negative association observed in most cohort studies. In the largest cohort study ever done on the use of tobacco and the occurrence of myeloma, 25 000 U.S. veterans were followed-up for 26 years (Heineman et al, 1992b). 582 myeloma deaths occurred in this study, but the relative risk of dying of myeloma was the same for both the smokers and those who had never smoked. Similarly, a smaller prospective cohort study of 17 633 male holders of Lutheran Brotherhood insurance, who completed a self-administered questionnaire about their use of tobacco in 1966, did not observe a significantly increased risk of death from myeloma among those who were smokers (Linet et al, 1992).

1.1.4.3 Alcohol

As mentioned previously, alcohol consumption has generally not been shown to be strongly associated with myeloma. This is based on a number of studies (Brown et al,

1992; Boffetta et al, 1989; Linet et al, 1987; Gallagher et al, 1983; Williams and Horm, 1977).

1.1.4.4 Food

Very few studies have assessed the relationship of food intake and myeloma risk. No association was found between intake of animal food, fried foods or fruits and vegetables with myeloma mortality in the study by Boffetta et al (1989).

1.1.5 Familial and Genetic Factors

Although myeloma is generally not regarded as an inherited disorder, the familial occurrence of the disease is well recognised. The role of genetic factors in the aetiology of myeloma is uncertain. Genetic factors may, in part, explain the increased incidence of myeloma in blacks compared to whites. Research on familial and genetic factors has focused on the following aspects, viz., i. Familial multiple myeloma, ii. Genetic marker studies, iii. Chromosomal abnormalities and iv. Molecular Genetics. In this review a brief mention will be made of these aspects.

1.1.5.1 Familial Myeloma

Familial myeloma was first suggested by Meyerding (1925) and Geschickter and Copeland (1928). The first well documented report of familial myeloma was described by Mandema and Wildervanck in 1954. Since then, there have been numerous reports of familial myeloma i.e. myeloma occurring in siblings and first-degree relatives (Herrinton

et al, 1995). A review of 104 cases occurring in 49 families has been reported by Olshan (1991). In his study, he found that the majority of cases occurred among siblings (66%), followed by parent-offspring (25%) cases. Other familial relationships include cousins, aunt-niece and aunt-nephew sets. Eriksson and Hållberg (1992), observed a relationship between first-degree family history of myeloma and myeloma occurrence (OR=5,6; 90% CI, 1,2-28). Furthermore, Olshan (1991), compared the age and sex distribution as well as the heavy and light chain isotypes of familial myeloma cases reported in the literature, with data from the SEER (Surveillance End Results and Epidemiology) program and the Mayo Clinic. The sex distribution was similar (slight male predominance). The mean age at diagnosis was 63 years for familial cases compared to 70 years for SEER cases. A slight excess of IgA was noted in familial cases, as was an elevated kappa : lambda ratio. An interesting immunogenetic and immunochemical study of two brothers with multiple myeloma and their relatives is reported (Grosbois et al, 1986). The brothers were diagnosed within 6 months of each other. Both had IgG kappa immunoglobulin isotypes, as well as identical HLA genotypes (A2;B12;DR4/A9;B27;DR2). Thirty four other families were tested and revealed no HLA genotype identity with the brothers. Nine family members were semi-identical for haplotype a and five for haplotype d. No other family members had a monoclonal gammopathy. The authors suggest that, based on the finding of a double immunochemical and immunogenetic identity in the two siblings, besides environmental factors, genetic factors may be involved in the pathogenesis of myeloma. The immunochemical similarity could also be a chance finding, as most cases of myeloma are IgG kappa.

Myeloma is also known to occur in twins. In Northern Ireland, a monozygotic twin pair developed myeloma concurrently, although the monoclonal immunoglobulin was different in both cases (McCrea and Morris, 1986). One twin's disease was IgA lambda, while the other twin had free kappa light chain myeloma. Two further twin pairs concordant for myeloma have also been reported. One of these was reported by Judson et al (1985), in England. Both brothers had IgG kappa myeloma and identical HLA types (A:2,W20;B:7,18;CW6,CW5). They were diagnosed within two years of each other. Interestingly, they lived and worked together unloading raw materials such as hemp and palm oil from ships at London docks. The third pair of twins had lived and worked separately since the age of 21. They were diagnosed eight years apart, one with IgG kappa and the other with kappa light chain myeloma (Commotti et al, 1987). In another family, two brothers had IgG kappa myeloma, while the sister had kappa light chain myeloma (Horwitz et al, 1985).

In addition, familial aggregation of disease has been noted. There appears to be an association between a history of autoimmune disease (rheumatoid arthritis), cancer (particularly of the lymphatic and haematopoietic system, breast, lung and genitourinary system) and degenerative central nervous system disease (Parkinson's disease and multiple sclerosis) in first-degree relatives, and the occurrence of myeloma (Grufferman et al, 1989; Linet et al, 1988; Bourguet et al, 1985; Isomaki et al, 1978). Bourguet et al (1985), in an attempt to confirm the familial occurrence of cancer, found 3 of 439 patients with myeloma compared to 4 of 1317 normal controls who reported myeloma in

their family. This study showed that a family history of cancer of any type resulted in a relative risk of myeloma of 1,4 (CI 1,1-1,8).

Average serum immunoglobulin levels were elevated in 200 first-degree relatives, but not spouses, of myeloma patients, compared with a control group of patients (Festen et al, 1977). In a study by Odeberg et al (1974), the distribution of immunoglobulin levels among relatives differed little from that of controls and very few markedly deviating levels were found. Thus, the study did not indicate a defective regulation of immunoglobulin production within myeloma families.

Myeloma has also been reported among spouses (conjugal myeloma), although it is uncommon (Kyle and Greipp, 1983; Brugiatelli et al, 1980; Kardinal, 1978; Pietruszka et al, 1976; Kyle et al, 1971). Kyle and Greipp (1983), reported two families in which successive spouses who lived in the same house developed myeloma. The significance of this occurrence is unknown, but it may suggest the possibility of a transmissible or environmental factor.

All the patients reported by Kyle et al (1971), were over the age of 50 years and had been married for between 6 and 41 years. The interval between the diagnosis of myeloma in one spouse and the other ranged from 1 month to 15 years, and no definite aetiological factors could be identified. There was also no concordance of the paraprotein type. More recently, a further report of myeloma occurring in a couple is documented (Keshava-Prasad et al, 1996). The couple developed the disease within a few months of each other, after being married for 49 years. This long period of being together raised the suspicion of exposure to a common aetiological factor, although none

could be identified. These reports of multiple cases of malignancy occurring in a family, without a clear Mendelian pattern of inheritance, suggests that the family members may be exposed to the same environmental hazard, although there has been no clear documentation in the reports of the agent involved.

On the other hand, the discovery that several affected family members have inherited identical HLA haplotypes (Loth et al, 1991; Grosbois et al, 1986; Blattner et al, 1980b), suggests that the tendency to develop these B-cell neoplasms may be inherited. In the case of Waldenstrom's macroglobulinemia (WM), the calculated LOD score (the log of the ratio of likelihood of linkage to no linkage) to test if the occurrence of WM and autoimmune manifestations in a family was segregating with the HLA region on chromosome 6 was 4,86. This score favours chromosomal linkage of a postulated susceptibility gene to the HLA complex (Blattner et al, 1980b).

1.1.5.2 Genetic Marker Studies

Two gene complexes are known to be relevant to immune regulation. The first is localized within the major histocompatibility complex (MHC), a biologically very important and well defined gene complex. Association between certain HLA (Human Leucocyte Antigen) types and susceptibility to a variety of diseases is well recognized. The second gene complex involved in immune regulation is linked to the Gm complex, which codes for allotypes of the IgG heavy chain. Km allotypes are located on the kappa light chain only.

Lymphocytes play a central role in immune function. As such, an ongoing search is being made for correlations of histocompatibility antigens with various lymphoproliferative disorders. Miller (1974), analyzed the HLA antigens of 77 adults with various forms of haematologic malignancy. He found an increase in HL-A5 and HL-A13 in patients with lymphoproliferative disorders (including myeloma), compared to a control group.

Several studies appear in the literature with respect to immunogenetic markers, and their similarities and differences in patients with myeloma (Riedel and Pottern , 1992; Olshan, 1991; Ludwig and Mayr 1982). With regard to HLA types in myeloma, many of the early studies found an association with the B locus. Significantly elevated risks have been reported in association with HLA-B5 (Festen et al, 1976; Mason and Cullen, 1975; Miller, 1974). Elevated, but non-statistically significant relative risks have also been reported (Jeannet and Magnin, 1976; Smith et al, 1974). However, there are studies which did not detect any association with HLA-B5 antigen (Ludwig and Mayr, 1982 ; Saleün et al, 1979; Van Camp et al, 1977; Bertrams et al, 1972). Ludwig and Mayr (1982) tested 68 myeloma patients and 3 000 controls for HLA types and noted no difference in HLA type; however, a combined, larger analysis of pooled data from the literature (379 cases and 5041 controls) showed an association between HLA-B5 among cases compared to controls. A significantly high frequency of HLA-B18 antigen has also been documented (Smith et al, 1974; Bertrams et al, 1972). More recently, associations have been found with the C locus, involving HLA-Cw5, HLA-Cw6 and HLA-Cw2 antigens (Leech et al, 1983; Pottern et al, 1992b). A significantly higher frequency of HLA-Cw2 has been noted in both American blacks and white men (Pottern et al, 1992b).

This study (of 46 black male cases and 88 controls) also demonstrated significantly higher gene frequencies in cases for Bw65 and DRw14, compared to controls, while white cases (85 males) had higher gene frequencies than controls (122 males) for A3 and Cw2, and blanks at the DR and DQ loci. The frequency of Cw2 in the black and white controls was similar. These findings suggest that the Cw2 allele, or a gene close to the C loci, confers susceptibility to the development of myeloma, but does not explain the higher risk among blacks. The authors also suggest that undefined class II antigens may play an aetiologic role. New molecular techniques, using genomic DNA, may lead to the identification of these alleles.

In American blacks, a strong association was found with the HLA-Cw5 antigen (4 of 22 cases compared to 2 of 138 controls; OR=15) (Leech et al, 1983). All four myeloma cases with the Cw5 antigen were males. In the same study, a relationship was also found with the Cw6 antigen (OR=6,5).

There is a paucity of data available regarding the involvement of the D locus and myeloma. Muylle (1982), in his study of 28 patients, found no association with HLA-DR antigens. Another study showed a higher proportion of HLA-DR positive cells and activated T cells being present in non-diseased American blacks compared to their caucasian counterparts (Tollerud et al, 1991).

Immunoglobulin allotypes, Gm and Km have also been studied for their possible association with myeloma. A strong association of G3m (g5) was found in American blacks with myeloma (29 patients and 160 controls, OR=15). (Leech et al, 1985). Based on this finding, the authors suggest that the G3m (g5) allotype may represent a marker of

inherited susceptibility to myeloma among blacks. However, the exact role of the allotype in the pathogenesis of myeloma is unknown.

More extensive immunogenetic studies are required in myeloma. These studies may help to define whether myeloma in a family occurs with a higher frequency in immunogenetically identical siblings than could be randomly expected and secondly, whether an abnormality of the immune response regulator genes located in the HLA D area could account for modification of immunoglobulin secretion in myeloma patients as well as in their relatives. The HLA D area (including the DR, DQ and DP loci), has previously been incriminated in other haematopoietic malignancies, especially those involving B-lymphocytes (Winchester et al, 1983).

1.1.5.3 Chromosomal Abnormalities

Cytogenetic information is limited in myeloma, as there is often difficulty in obtaining analyzable metaphases, because of the low proliferative activity of plasma cells. Abnormal karyotypes are found in approximately 30-50% of cases (Luc Lai et al, 1995; Sawyer et al, 1995; Weh et al, 1993; Gould et al, 1988; Dewald et al, 1985), while flow cytometric analysis of nuclear DNA content of G1/0 cells has led to the demonstration of an aneuploid myeloma cell population in approximately 30-80% of the patients (San Miguel et al, 1995; Barlogie et al, 1989). Based on the flow cytometry derived aneuploidy data and fluorescence in situ hybridization (FISH) analysis, it is likely that conventional cytogenetics still fails to detect about 40% of the cytogenetic abnormalities. Flow cytometric and FISH analysis indicate the presence of cytogenetic abnormalities in

the vast majority of patients (approximately 80%-90%), irrespective of their disease status (Tabernero et al, 1996; Drach et al, 1995; Barlogie et al, 1989). Based on these findings, it is likely that the majority of normal karyotypes in myeloma patients are derived from normal haemopoietic cells and not from the myeloma clone. Previously treated and relapsing patients have a higher frequency of chromosomal abnormalities (35-60%), compared with 20-35% in newly diagnosed patients (Lai et al, 1995; Sawyer et al, 1995; Weh et al, 1993; Gould et al, 1988; Dewald et al, 1985). The higher incidence of abnormal cytogenetics in advanced disease probably reflects an increased proliferative rate with disease progression (Drewinko et al, 1981). Flow cytometry and FISH analysis have also demonstrated aneuploidy in 50%-60% of patients with monoclonal gammopathy of undetermined significance (MGUS) (Drach et al, 1995; Zandecki et al, 1995; Latreille et al, 1982). Thus, it appears that the processes leading to karyotypic instability begin in MGUS, progress substantially in overt myeloma, and continue to progress throughout the entire course of the disease.

In myeloma, cytogenetic abnormalities may be structural or numerical. The complexity of most myeloma karyotypes are consistent with a long subclinical course during which multiple aberrations may occur. There is no specific chromosomal abnormality linked to myeloma. However, the most characteristic numerical abnormalities are monosomy 13, and trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. Non-random structural abnormalities most frequently involve chromosome 1 and 14. Less commonly, other chromosomes such as 8, 12, 16, 17 and 18 are associated with the disease (Tricot et al, 1995; Sandberg, 1990; Dewald et al, 1985; Lewis and MacKenzie, 1984).

Table 1.1 Cytogenetic characteristics in 155 myeloma patients

KARYOTYPE	%
NORMAL DIPLOID	51
INEVALUABLE	10
ABNORMAL	39
MULTIPLE TRISOMIES (>3)	17
-13/13q-	14
14q	11
11q	9
1q	9
1p	9
6q	3

Adapted from Tricot et al, 1995.

A breakdown of cytogenetic abnormalities found in a study of 155 patients by Tricot et al (1995), is shown in Table 1.1. In a more recent review, Feinman et al (1997), analyzed the karyotypes of 492 myeloma patients who received high-dose cyclophosphamide to mobilize peripheral blood stem cells as part of a double autotransplant procedure after regimens containing high-dose melphalan. Only karyotypes obtained prior to the first autotransplant were taken into account. Of the 492 patients, 153 (31%) had a normal karyotype, 133 (27%) were abnormal and 206 (42%) were inevaluable. A normal karyotype was based on at least two normal cytogenetic analyses. Patients with 0 or 1 cytogenetic analysis or with less than 20 metaphases available for evaluation were considered inevaluable. Of the 133 patients with abnormal karyotypes, 73 (55%) had three or more trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. 57 patients (43%) had an abnormality of chromosome 13, which was either a complete or partial loss of the chromosome, 38 patients (28,5%) had abnormalities involving 1q and

33patients (25%) had abnormalities involving chromosome 14. 32 patients (24%) had involvement of chromosome 1p, 28 patients (21%) 11q and 20 patients (15%) 6q respectively (Feinman et al, 1997). A number of patients had combinations of these abnormalities (see Table 1.2).

Table 1.2 Cytogenetic characteristics in 492 myeloma patients

KARYOTYPE	%
NORMAL	31
INEVALUABLE	42
ABNORMAL	27
MULTIPLE TRISOMIES (>3)	55
-13/13q	43
14q	25
11q	21
1q	28.5
1p	24
6q	15

Adapted from Feinman et al, 1997

B lymphoproliferative disorders are often associated with abnormalities of the light chain immunoglobulin loci on chromosome 2 (kappa) and 22 (lambda) and heavy chain locus on chromosome 14. A review of the cytogenetic literature from the 1960s through to the 1980s, revealed 453 mycloma patients of whom 124 had complex karyotype abnormalities. Of these 124 patients 32% had various chromosomal abnormalities involving chromosome 14 (Dewald et al, 1991). These include 14q+ (Wurster-Hill et al, 1973) and translocations such as t(8;14)(q24;q32); t(11;14)(q13;q32) and t(14;18) (Dewald et al, 1991; Gould et al, 1988; Gahrton et al, 1980). In earlier studies, 14q

abnormalities were associated with a poor prognosis (Sawyer et al, 1994), but Tricot et al (1995), have recently shown that this is entirely due to cases with a t(11;14). The poor prognosis in patients with 11q abnormalities may be related to abnormalities of bcl-1 expression which is well documented in patients with t(11;14)(q13;q32), in mantle cell-derived lymphomas (Raffeld and Jaffe, 1991). Fiedler et al (1992), analyzed 17 patients with myeloma for the presence of bcl-1 alterations. Karyotype analyses of 14 patients revealed 3 patients with abnormalities in 11q13, two of them with t(11;14), and one with deletion 11q13. However, Southern blot analyses did not reveal any abnormalities within the bcl-1 locus. In B-cell tumours, e.g. Burkitt's lymphoma and mantle cell lymphoma, oncogenes are dysregulated by translocation into the J region of IgH locus, whereas in myeloma, detailed molecular analyses of the reciprocal 11;14 breakpoints and the productive switch recombination breakpoint in the myeloma cell line U266 clearly indicate that, at least in this one example that has been fully characterized, the aberrant 11;14 rearrangement resulted from an error occurring at the time and is related to the process of productive isotype switch recombination (Gabrea et al, 1999; Bergsagel et al, 1996).

The t(4;14) and t(14;16) are characteristic in that they have only been described in myeloma samples; similarly, a t(11;14) translocation occurring in the switch region has only been described in myeloma samples. Many of these translocations are not detected by conventional karyotypic analysis, although recent analyses using reverse transcription-polymerase chain reaction (RT-PCR) (Chesi et al, 1998), multicolor spectral karyotypes (Sawyer et al, 1998), and dual-color interphase fluorescent in situ hybridization (Avet-

Loiseau et al, 1998), indicate that these translocations occur at a similarly high frequency in primary patient material.

A poor prognosis associated with abnormalities of chromosome 11q was also noted in the review of Feinman et al (1997). Calasanz et al (1997), noted further that hypodiploidy and 22q11 rearrangements at diagnosis were associated with a poor prognosis in patients with myeloma.

Partial or complete deletions of chromosome 13 are also associated with a poor prognosis (Tricot et al, 1995). These unfavourable karyotypes (-13/13q or 11q) were associated with a significantly higher incidence of IgA myeloma, elevated beta 2 microglobulin and advanced age (Tricot et al, 1995). Patients with a normal karyotype had a median event free survival (EFS) and overall survival (OS) of 43 and greater than 50 months respectively, while patients with a partial or complete deletion of chromosome 13 had median EFS and OS of 21 and 29 months respectively. Those who had 11q abnormalities had an EFS and OS of 20 and 21 months (p=0,0001), while the patients who had combined 11q and -13/13q had the worst prognosis, with EFS and OS of 11 months and 12 months respectively (Tricot et al, 1995). Two recent reviews looking at the prognostic value of karyotypes in myeloma have confirmed the poorer prognosis associated with monosomy 13 (Pérez-Simón et al, 1998; Seong et al, 1998). Pérez-Simón et al (1998), in their study of numerical chromosome aberrations by FISH analysis, showed that monosomy 13 (as assessed by deletion of the Rb gene) was associated with a shorter survival, compared to trisomies of chromosome 6, 9 and 17 which are associated with prolonged survival in myeloma patients. They recommend

FISH analysis as a reliable technique that may afford accurate information on numerical changes, overcoming the problem of conventional cytogenetics, and more specifically, FISH evaluation of chromosome 6, 9, 13 and 17 on all patients with myeloma at diagnosis, because of the prognostic value of their findings.

19q13 abnormalities have also been reported in myeloma and plasma cell leukaemia (Lai et al, 1995; Mitelman, 1991; Yip et al, 1990). In addition, t(1;16)(p11;p11) has been documented in two patients with myeloma (Flactif et al, 1994).

1.1.5.4 Molecular Genetics

Cellular oncogenes are normal cellular genes that are important for the growth and differentiation of cells. When activated, the genes lead to transformation of a cell. The activation can occur via different mechanisms, viz., chromosomal translocations, point mutations, integration of a viral promoter or enhancer sequence in the vicinity of a cellular oncogene or gene amplification. These mechanisms result either in the enhanced expression of the oncogene or a structurally altered product. Dysregulation of oncogenes and loss or inactivation of tumour suppressor genes controlling cellular proliferation, growth arrest, and apoptosis contribute to the pathogenesis of most malignancies, including myeloma.

The development of myeloma appears to be a multistep process with alterations in the IL-6/ras pathway, dysregulation of c-myc and bcl-2 proteins and loss of tumour suppressor gene products such as retinoblastoma and p53.

c-myc is a transcription factor that regulates transformation, proliferation and apoptosis (Ryan and Birnie, 1996). c-myc is expressed in proliferating cells and is closely associated with cell cycle progression. c-myc has been shown to interact with pRB (retinoblastoma gene product) and RB-related p107 and abrogates pRB and p107-induced growth arrest (Beijersbergen et al, 1994; Hateboer et al, 1993). c-myc may be stimulated to induce cell proliferation by cytokines such as IL-2, while in the absence of appropriate growth factors, overexpression of c-myc has been shown to induce p53 mediated apoptosis in fibroblasts and myeloid cells (Ryan and Birnie, 1996). At a molecular level, c-myc is involved in the transcriptional activation of genes involved in cell cycle progression, such as cyclin D1 and in the transcriptional repression of genes involved in growth arrest and differentiation.

Alterations in the c-myc locus, with concomitant increased expression of mRNAs in tumour cells has been demonstrated in myeloma (Nobuyoshi et al, 1991; Selvanayagam et al, 1988; Gazdar et al, 1986, Sumegi et al, 1985). c-myc alterations are infrequent in myeloma: increased levels were found in 9/37 patients: 2 had c-myc gene rearrangements (Selvanayagam et al, 1988), compared to other B-cell tumours such as Burkitt's lymphoma, in which c-myc gene rearrangements are common. Also, the cloned, rearranged DNA appears to be entirely derived of chromosome 8, indicating a novel mechanism of c-myc activation, which is different from that in Burkitt lymphomas. The elevated c-myc mRNA expression in patients with myeloma is correlated with higher levels of cellular proliferation.

Bcl-2 is a cytoplasmic membrane protein. The bcl-2 protein functions on the inner cell membrane of the mitochondria. It prolongs the survival of progenitor and terminally differentiated cells (Yang and Korsmeyer, 1996). Bcl-2 prevents apoptosis induced by a wide variety of agents, such as glucocorticoids (dexamethasone), cytotoxic drugs (topoisomerase II inhibitors) etc. Furthermore, myeloma cells are protected against dexamethasone-induced apoptosis by insulin-like growth factors (Xu et al, 1997). The physiologic role of endogenous bcl-2 is essential for T and B cell differentaition and the maintenance of B-cell memory (Yang and Korsmeyer, 1996). Although the precise mechanism by which bcl-2 blocks programmed cell death is still unclear, it has been concluded that bcl-2 suppresses apoptosis in cycling cells and prolongs the survival of noncycling cells. Overexpression of bcl-2 protein has also been observed in multiple myeloma (Brown et al, 1994; Durie, 1991; Durie et al, 1991). Approximately 75% of patients studied showed aberrant increased expression of the bcl-2 oncogene. Unlike in most follicular lymphomas, there was neither a correlation with obvious cytogenetic abnormalities such as t(14;18), nor insertion of the bcl-2 gene into the immunoglobulin light chain locus. Other proteins also belong to the bcl-2 family. Bcl-2 and bcl-$_{XL}$ prevent apoptosis (Boise et al, 1993; Nunez et al, 1990), whereas bax and an alternatively spliced bcl-x product, bcl-$_{XS}$, promotes cell death (Boise et al, 1993; Oltvai et al, 1993). When bcl-2 heterodimerizes with bax, apoptosis is accelerated, while homodimerization results in suppression of apoptosis (Oltvai et al, 1993). Similarly, excess amounts of bcl $_{XS}$ antagonize the function of bcl-$_{XL}$ (Minn et al, 1996). Therefore, a critical balance between bcl-2, bax, and bcl-$_{XL/S}$ molecules may determine the fate of cells in response to

cytotoxic agents or a pro-apoptotic natural environment. Recently, it has been shown that CD40 and CD95 (Fas or Apo1/Fas) may also be implicated in the induction of apoptosis in myeloma (Bergamo et al, 1997). IL-6 has been implicated in the resistance of myeloma cells to programmed cell death pathways including Fas-mediated apoptosis. Catlett-Falcone et al (1999), recently reported that one STAT (signal transducer and activator of transcription) family member, STAT3, is constitutively activated in bone marrow mononuclear cells from patients with myeloma. Activation of STAT3 results in over-expression of the anti-apoptotic protein bcl_{XL}. Blocking IL-6 receptor signalling from Janus kinases to the STAT3 protein inhibits bcl_{XL} expression and induces apoptosis, demonstrating that STAT3 signalling is essential for the survival of these tumour cells. These findings provide evidence that STAT3 signalling contributes to the pathogenesis of myeloma by preventing, at least in part, Fas induced apoptosis.

The ras family of genes encodes a large family of plasma membrane-associated GTP-binding proteins. These proteins are involved in signal-transduction pathways, which are critical for cell proliferation and differentiation. GTP-binding proteins are activated when GTP-bound and inactivated when GDP-bound (Bourne et al, 1991). Three human ras proto-oncogenes have been identified: K-, H- and N-ras. Activated ras transduces signals from cell surface receptors and non-receptor associated tyrosine kinases (e.g. bcr-abl) to its downstream target, raf, a serine-threonine kinase, which in turn phosphorylates the mitogen-activated protein kinase (MAPK/MAP) and mammalian MAPK/Erk kinase (MEK) (Khosravi-Far and Der, 1994). In T cells, ras has been shown to participate in

the protein kinase C (PKC) dependent and independent signalling pathways (Downward, 1992).

Activation of the ras protein results in altered gene expression. Ras oncogene mutations have been reported in 49% of 160 newly diagnosed myeloma patients, with N-ras being the most frequent (Liu et al,1996). Point mutations have been detected in both the K-ras and N-ras oncogene. A significantly shorter survival and increased tumour burden has been associated with mutations in patients harbouring the K-ras gene only. The median survival for K-ras mutations was 2 years, versus 3,7 years for N-ras or no mutations (Liu et al, 1996). The percentage of point mutations was higher in patients with more advanced disease, as well as those with plasma cell leukaemia (Corradini et al, 1993; Neri et al, 1989). The codons involved were similar to those observed in other types of human tumours, including codons 12, 13, 61 for both K-ras and N-ras . Some ras mutations are acquired during the course of the disease, implying that the mutations may not be the initiating tumorigenic event, but rather, may influence disease progression.

Inactivation of tumour suppressor genes may also contribute to malignant transformation (Weinberg, 1989). The role of the Rb-1 (Retinoblastoma-1) gene in myeloma will briefly be elaborated upon. One may speculate that the inferior outcome of patients who have deletions of the long arm of chromosome 13 is associated with deletion of the Rb-1 gene, located on 13q14. The retinoblastoma gene product (pRB), a 110 kDa nuclear matrix protein, represents the prototype tumour suppressor gene. pRB has been shown to inhibit apoptosis and facilitate differentiation (Haas-Kogan et al, 1995; Lee EY-HP et al, 1994; Slack et al, 1993). Whether a cell will undergo growth arrest in G1 or traverse

the G1/S boundry and proliferate depends on the phosphorylation status of pRB. In early and mid G1, pRB is hypophosphorylated, whereas in late G1, pRB becomes hyperphosphorylated on serine and threonine residues by cyclin-dependent kinases (CDKs) (Weinberg, 1995). During the S, G2 and M phases the hyperphosphorylated status is maintained. Growth stimulatory signals such as mitogens and growth factors induce the accumulation of G1 cyclins, D and E in early to mid G1; which in turn regulate the catalytic activities of CDK2 and CDK4 or CDK6 respectively. In contrast, growth inhibitory polypeptides such as transforming growth factor (TGF)-β, and antimitogenic agents have been found to inhibit pRB phosphorylation by downregulating CDK function.

PRAD1/cyclin D1 stimulates phosphorylation of Rb protein and thereby inactivates its growth suppressive properties (Ewen et al, 1993). Defective or absent Rb protein is advantageous for cell proliferation and may initiate or contribute to progession of cancer. Deletion of the Rb-1 has been observed in more than 50% of myeloma patients (using the FISH tecnhique) (Dao et al, 1994). Loss of the Rb protein was observed in 35% of primary cases of myeloma and plasma cell leukaemia and inactivation of the Rb-1 gene is associated with more aggressive disease (Corradini et al, 1994). A model of tumorigenesis in lung carcinoma has been proposed, in which overexpression of PRAD1/cyclin D1 is an early event, followed by Rb protein loss (Shapiro et al, 1995). Therefore, it is conceivable that increased phosphorylation of decreased amounts of Rb protein confer further loss of growth control. It has also been suggested that another tumour suppressor gene may be present on chromosome 13 and that the Rb-1 gene

deletion may be assuming an 'innocent bystander' role, although it is considered to be the most likely target of allelic deletions in 13q (Dodson et al, 1994). A region (locus D13525) located telomeric to the Rb-1 gene (DBM gene-deleted in B-cell malignancy gene) has been identified, which is probably homozygously deleted in chronic lymphocytic leukaemia (CLL) (Liu et al, 1993), which is another B-cell lymphoproliferative disorder with a high frequency of monoallelic Rb-1 deletions (Stilgenbauer et al, 1993). More recently, frequent somatic deletion of the 13q12.3 locus, encompassing BRCA-2, has been reported in CLL (Garcia-Marco et al, 1996). BRCA-2 is probably the candidate gene whose somatic inactivation could play a role in initiation and progression of B-CLL as well as myeloma.

The p53 gene, a tumour suppressor gene, is altered frequently through deletion or point mutations in malignant tumours. It is the most commonly mutated gene in human malignancies, with the majority of missense point mutations occurring in the amino acid region spanning exons 5 to 9 (Hollstein et al, 1990). p53 gene mutations have been detected by several investigators in myeloma (Neri et al 1993; Willems et al, 1993). Willems et al (1993), found mutations of p53 in 7/19 patients, while Neri et al (1993), observed mutations in 7/52 patients. p53 mutations occur more commonly with advanced stage disease, aggressive and leukaemic forms of the disease and are associated with a poor prognosis. Recently, Avet-Loiseau et al (1999), showed that p53 deletion is not a frequent event in myeloma. Using FISH, they found only 7/79 (9%) patients (with advanced stage myeloma) with a p53 deletion. Upon reanalysis of their findings, they confirmed the low incidence found initially. They suggest that poor hybridization

efficiency (a feature of malignant plasma cells rather than other bone marrow cells) may lead to a false p53 deletion.

Rb-1 and p53 both repress interleukin-6 (IL-6) (Santhanam et al, 1991): a putative growth factor in myeloma (Klein et al, 1995). p53 also inhibits expression of bcl-2, an inhibitor of apoptosis in B-cell malignancies including myeloma (Korsmeyer, 1992).

With regard to oncogenes in myeloma, the most significant pattern to emerge in cross-sectional studies was the fact that stable disease was associated with the overexpression of either an oncoprotein or a tumour suppressor gene product, while progressive disease was associated with the overexpression of both an oncoprotein and a tumour suppressor gene product. This suggests that disease progression in myeloma, as in colorectal tumours, requires the dysregulation of both an oncogene and a tumour suppressor gene (Joshua et al, 1994).

Although the pathogenesis of myeloma is still unclear, a multistep transformation process is likely. A hypothetical model may be conceived based on numerous studies which have explored and examined the molecular biology of the disease (Bergsagel et al, 1999; Hallek et al, 1998; Feinman et al, 1997; Bersagel et al, 1996). The oncogenic events in myeloma either occur after or do not interfere with the normal maturation process that generates the long-lived plasma cell. Like a long-lived plasma cell, a myeloma cell has undergone three developmentally regulated changes in the DNA structure of the IgH and IgL loci, including productive V(D)J recombination and somatic hypermutation of the IgH and IgL genes, and productive IgH switch recombination to another IgH isotype. It

is possible that errors in one or more of these processes may result in the genetic changes that contibute to the malignant process.

The malignant transformation process may be initiated by an Ig gene translocation related to Ig recombination processes, that results in ectopic expression (dysregulated expression) of an oncogene such as cyclin D1, c-myc, FGFR3, c-maf and MUM1(IRF4), caused by the juxtaposition to strong regulatory sequences of the IgH locus and resulting in immortalization of the malignant clone.

Dysregulated c-myc levels may be the first growth stimulatory signal in the development of myeloma. c-myc, together with p53, counteracts pRB and p107-mediated growth arrest signals and thus stimulates cell proliferation. Additionally, the myeloma cells may be susceptible to cytokine-deprived or growth factor-deprived apoptosis. There is early acquisition of chromosomal instability, with multiple trisomies present even in MGUS and frequent monosomy 13 in myeloma.

The second proliferative signal may be activation of the ras/MAP kinase pathway, by secretion of IL-6 and related cytokines from the bone marrow stromal cells in response to adhesion of myeloma cells. Activation of the ras/MAP kinase pathway prevents c-myc induced apoptosis. Activation of ras results in upregulation of bcl-2 and other antiapoptotic factors that prolong survival of myeloma cells. Eventually, myeloma cells become independent of extensive growth stimuli owing to ras mutations. The ensuing constitutive ras activation results in higher expression of bcl-2 and endogenous IL-6 secretion by myeloma cells. The hypermutational process onto ras or a tumour

suppressor gene on chromosome 13, result in selection of a single clone for malignant expansion.

In the advanced stages of the disease, loss of p53 function occur, leading to genomic instability as evidenced by complex karyotypic abnormalities and translocations. The secondary IgH translocation to a variety of loci occur by mechanisms unrelated to physiologic Ig recombination processes, that may reflect the presence of a more general genomic instability. Further mutations, like p53, lead to stroma-independent growth, and escape from the bone marrow microenvironment (Bergsagel et al, 1999; Hallek et al, 1998; Feinman et al, 1997). The progressive genetic events are summarised in Figure 1.2.

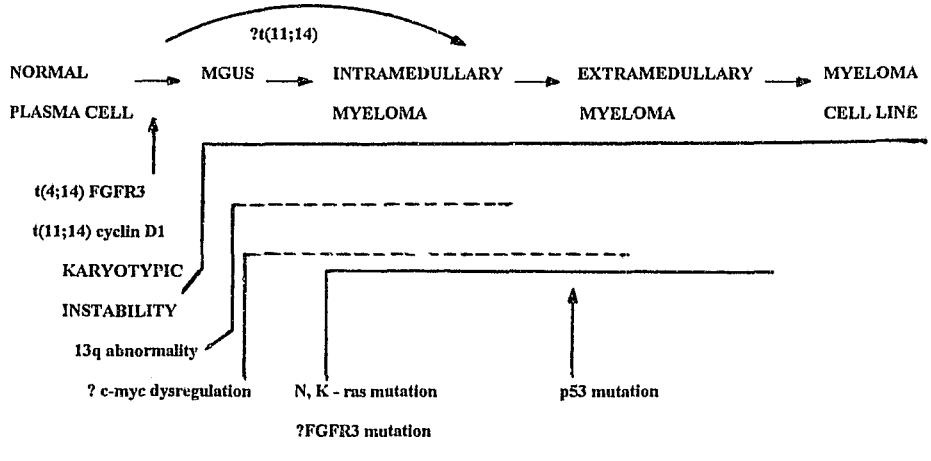


Figure 1.2 Summary of progressive genetic events in multiple myeloma (Adapted from Hallek et al, 1998).

1.1.6 Pre-existing medical conditions / chronic antigenic stimulation

Chronic antigenic stimulation (CAS) of the immune system has been suggested as a risk factor in the development of myeloma. This is based on the assumption that antigenic stimulation in a susceptible host is the first hit, of the so called 'two-hit hypothesis', giving rise to a benign monoclone. The second hit is postulated to be a mutagenic or transforming event that gives rise to myeloma from the expanded monoclonal B cell population (Salmon and Seligmann, 1974).

The development of plasma cell tumours in close proximity to chronic caecal irritation in C_3H mice (Pilgrim, 1965; Dunn, 1957) and the development of myeloma after injection of Freund's adjuvants, mineral oil and plastics in BALB/c mice (Potter M, 1985; Potter 1962; Potter and Boice 1962; Potter and Robertson, 1960), is evidence in support of the role of CAS. In BALB/c mice, granulomatous lesions comprised of plasma cells, lymphocytes and histiocytes develop initially at the site of injection. Concomitantly, a diffuse polyclonal hypergammaglobulinaemia is found (Farhangi and Osserman, 1978). After 6 to 12 months, 60-70% of these mice develop plasmacytomas that produce a paraprotein in the serum and light chains in the urine (Farhangi and Osserman, 1978). Other studies suggest that the development of plasma cell tumours in BALB/c mice results from the combined stimulation of the reticuloendothelial system by chemical stimulants and intestinal bacteria (Potter M, 1973; Potter M, 1972; Potter and Leon, 1968). In these studies, the paraprotein isotype was mainly IgA and possessed in many cases antibody activity to antigens of the gut flora, dietary components and other determinants (Potter M, 1977; Potter M, 1973). In other animal models, such as Aleutian

mink and New Zealand Black mice, the development of monoclonal plasma cell disorders is preceded by autoimmune disease, widespread lymphoid hyperplasia and polyclonal hypergammaglobulinaemia (Farhangi and Osserman, 1978; Mellors, 1969; Porter et al, 1965; Potter M,1962). Based on these animal models, it appears that a combination of genetic factors and protracted chemical, bacterial and viral stimulation of the immune system leads ultimately to the development of plasma cell tumours (Farhangi and Osserman, 1978). Circumstantial evidence exists that similar mechanisms may be operative in the pathogenesis of human plasma cell disorders.

A different viewpoint has also been proposed. Evidence has accumulated which shows that the malignant transformation in myeloma occurs at an earlier level in the lymphocyte ontogeny i.e. at the level of a pre-B or stem cell (Barlogie and Epstein, 1990); and that these cells are not stimulated by antigen. Nevertheless, irrespective of the role of CAS, there is growing evidence that CAS could facilitate progression of disease. This may occur via the cytokine interleukin-6 (IL-6). IL-6 is a potent stimulator of B-cell differentiation and is essential for the survival and growth of myeloma cells (Klein et al, 1989; Kawano et al, 1988). IL-6 was initially found to be a growth factor for myeloma cells (Klein et al; 1989; Kawano et al, 1988), but recently it has also been shown to promote the survival of myeloma cells by preventing spontaneous or dexamethasone induced apoptosis (Hardin et al, 1994). These data from in vitro studies suggest that IL-6 promotes both tumour growth and resistance to dexamethasone in vivo. IL-6 appears to be a paracrine growth factor (produced by cells in the microenvironment of the bone marrow), rather than an autocrine growth factor (Klein et al, 1989; Kawano et al, 1988).

Myeloma cells can however stimulate stromal cells and bone cells to release large amounts of IL-6 (Carter et al, 1990). Overproduction of IL-6 by cells in the marrow microenvironment leads to an increased production of the cytokine in patients with myeloma. Myeloma cells express specific receptors for this cytokine. The IL-6 receptor has two polypeptide components: the α chain (composed of the glycoprotein subunit gp80, or IL-6 receptor α) and the β chain, a transducer element (gp130). Myeloma cells shed the soluble form of IL-6 receptor α, which can amplify the response of myeloma cells to IL-6 (Gaillard et al, 1993). IL-6 receptor α is present in increased amounts in the serum of patients with myeloma, especially those with a poor prognosis (Bataille et al, 1992b). IL-6 is produced by many cell types in response to a number of stimuli including bacterial products, viruses and trauma (Wolverkamp and Marquet, 1990). IL-6 production has also been noted in other diseases, such as rheumatoid arthritis, systemic lupus erythematosus, trauma, acute infectious neural disease, cardiac myxoma and transplantation (Wolverkamp and Marquet, 1990). These conditions could be regarded as risk factors for the development of myeloma via IL-6 production.

Another pathway by which CAS could be associated with myeloma is through production of a monoclonal protein (M-protein). M-component (or M-protein) production may be transient or chronic. Transient production occurs normally in response to trauma, drugs and infections with specific antigens (Haas et al, 1990; Merlini and Aguzzi, 1988). In the study by Haas et al (1990), increased levels of monoclonal protein were found to be present in patients with leishmaniasis (16/20) and

cytomegalovirus (8/18), but not in those with echinococcus (1/18), infectious mononucleosis (0/30) or healthy control subjects (1/39).

Chronic production can occur in response to rheumatoid arthritis (Zbigniew et al, 1967) and ankylosing spondylitis (Renier et al, 1992), infections (e.g. tuberculosis, human immunodeficiency virus) (Turbat-Herrera et al, 1993), malignancy (e.g. breast, colon and prostate carcinoma; lymphoma), neurological and dermatological diseases, chronic liver disease, post renal transplant immunosuppression and a number of miscellaneous conditions (Passweg et al, 1996; Bladé and Kyle, 1995; Merlini and Aguzzi, 1988; Saleün et al, 1982).

In approximately 60-90% of persons identified with elevated M-components in community-based surveys, the elevation is chronic and unexplained. This so called benign unexplained monoclonal gammopathy is referred to as MGUS (Monoclonal Gammopathy of Undetermined Significance) (Kyle, 1984; Saleün et al, 1982). MGUS is characterized by the presence of a serum monoclonal protein (M-protein) of less than 3 g/dl, fewer than 10% plasma cells in the bone marrow, no or only small amounts of an M-protein in the urine (Bence Jones protein), absence of lytic lesions, no anaemia, hypercalcaemia and renal insufficiency, and stability of the M-protein. There is also no evidence of myeloma, macroglobulinaemia, amyloidosis or other disorders associated with a monoclonal gammopathy.

The prevalence of MGUS, i.e. the number of cases in a defined population at a particular time is approximately 20 times greater than myeloma (Axelsson, 1986). MGUS is found in approximately 3% of persons older than 70 years and in 1% of those above 50 years

of age (Saleün et al, 1982; Kyle et al, 1972; Axelsson et al, 1966). Of 1,200 patients 50 years or older, who were residents of a small Minnesota community with a cluster of myeloma, 15 (1,25%) had an M-protein (Kyle et al, 1972), and 303 (1,7%) of 17,968 adults 50 years or older in Finistère, France, had an M-protein (Saleün et al, 1982). In Sweden, among 6,995 persons older than 25 years, the overall rate of an M-protein was 1% (Axelsson et al, 1966). An M-protein was detected in 1,2% of 73,630 hospitalized patients in the United States (Vladutiu, 1987) and in 0,7% of 102,000 serum samples from a general hospital in Italy (Malacrida et al, 1987). In another study, 10% of 111 persons older than 80 years had an M-protein (Crawford, 1987), and in a further study, 23% of 439 patients between the ages of 75 and 84 years had an M-protein (Ligthart et al, 1990). In contrast to the latter two studies in elderly persons, the incidence of monoclonal gammopathy is less in elderly Japanese patients (Bowden et al, 1993). With the exception of the latter study, these studies are consistent with respect to the demonstration of an age related increase in the prevalence of MGUS, similar to that observed in myeloma. MGUS is also 1,5-2 times more likely to occur in males than females of a similar age group (Saleün et al, 1982; Kyle et al, 1972; Axelsson et al, 1966). In addition, the French survey observed the prevalence of MGUS to be 30% higher in farmers than persons in other occupations after adjustment for age and sex (Saleün et al, 1982).

In two studies, the incidence of M-protein was higher in the African-American population than in caucasians (Schecter et al, 1990; Singh et al, 1990). The study of Schecter et al (1990), demonstrated a 1,7 fold increase in the frequency of benign

paraproteinemia in blacks compared to caucasians. The paraproteinemia was also noted at an earlier age in the black patients, similar to the age distribution of myeloma in black patients as compared to caucasians (Blattner, 1980a). The subjects of his study were hospitalized patients, and, as such, the increased prevalence of paraproteinemia may have reflected factors related to acute or chronic illness. The authors therefore suggest that the factors playing a role in the increased susceptibility of black individuals to develop myeloma may also result in an increased propensity to form plasma cell clones of limited growth potential which result in the syndrome of benign paraproteinemia.

Progression of MGUS to myeloma and macroglobulinaemia has been examined in a small community-based survey, in two studies of persons with MGUS detected during routine laboratory testing, and in five series of haematology patients referred for evaluation of serum and urine proteins.

The community-based survey was of 6,995 residents of the Värmland district of Sweden. Using paper electrophoresis, 64 persons were identified with MGUS. During a 20 year follow-up, seven (11%) had progression of their benign monoclonal gammopathy; however, three patients with an increase in the M-protein and a fourth patient with a large serum IgA κ protein and Bence Jones proteinuria were still alive without requiring chemotherapy at the time of the report (Axelsson, 1986). Of the two studies of persons with MGUS detected during laboratory testing, one was the University of Barcelona study. MGUS was identified during routine laboratory testing with cellulose acetate in 128 patients. At follow-up (median of 56 months), malignant disease developed in 13 patients (10,2%). The actuarial probability for development of malignant disease was

8,5% at 5 years and 19,2% at 10 years (Bladé et al, 1992). The second study was conducted among blood ' nors at a French transfusion centre. Of 20 patients with asymptomatic monoclonal gammopathy followed for 3 to 14 years, 2 had myeloma and 2 developed macroglobulinaemia (Fine et al, 1979).

A number of studies in haematology patients also confirm progression of MGUS to myeloma or related disorders (Kyle, 1997; Ucci et al, 1993; Manthorne et al, 1988; Carter and Tatarsky, 1980; Pick et al, 1977; Kyle, 1995). At the Mayo Clinic, 241 persons with MGUS have been followed for 24 to 38 years. The group comprised of 140 male and 101 female patients (median age at diagnosis, 64 years). The initial M-protein level ranged from 0,3 to 3,2 g/dl (median value, 1,7g/dl). The heavy-chain type was IgG in 73%, IgM 14%, IgA 11% and biclonal in 2%. The light chain was κ in 62% and λ in 38%. 15 patients had Bence Jones proteinuria, but the amount of urinary light chain was more than 1 g per 24 hours in only 3 patients. The levels of uninvolved immunoglobulins were reduced in 29%. The percentage of plasma cells ranged from 1-10% (mean 3%) (Kyle, 1997). The 241 patients in this study were classified into 4 groups (see Table 1.3). Group 1 was made up of those individuals in whom the M-component remained stable (30 patients, 12%). In group 2, the M-component increased to 3g/dl or more, but no chemotherapy was required for myeloma or macroglobulinaemia (23 patients, 10%). 126 patients (52%) constituted group 3. These individuals did not develop a malignant plasma cell or lymphoproliferative disorder, but died of unrelated diseases, most commonly cardiac or cerebrovascular disorders. In group 4 there were 62 patients

(26%), in whom myeloma, macroglobulinaemia, amyloidosis or a malignant lymphoproliferative disorder developed.

Table 1.3 Course of 241 patients with Monoclonal Gammopathy of Undetermined Significance

Group	Description	At follow up 24-38 years later Number	%
1	No substantial increase of serum or urine monoclonal protein (benign)	30	12
2	Monoclonal protein > 30g/l but no myeloma or related disease	23	10
3	Died of unrelated causes	126	52
4	Development of myeloma, macroglobulinaemia, amyloidosis or related disease	62	26
Total		241	100

Adapted from Kyle, 1995.

The actuarial rate of development of serious disease was 16% at 10 years, 33% at 20 years and 40% at 25 years. Of the 62 patients, 42 (68% of group 4; 17,4% of the whole group) had myeloma. The interval from recognition of the M-protein to the diagnosis of myeloma ranged from 2 to 29 years (median - 10 years). In 7 patients, myeloma was diagnosed after a period of more than 20 years from detection of the serum M-protein. The median duration of survival after the diagnosis was 33 months. Development of myeloma varied from a gradual increase of the M-protein to an abrupt increase. At follow-up assessments, the size of the M-protein (>3g/dl), haemoglobin value (<12g/dl), percentage of bone marrow plasma cells (>25%), amount of light chain excretion,

presence of hypercalcaemia or renal insufficiency and presence of lytic lesions are often helpful in differentiating MGUS from myeloma. The plasma cell labelling index and the presence of circulating plasma cells in the peripheral blood are indicators of active disease; however, there are no findings at the diagnosis of MGUS that reliably distinguish patients who will remain stable from those in whom a malignant condition will develop. Thus, the physician must perform serial measurements of the M-protein in the serum and periodic evaluation of the pertinent clinical and laboratory features to determine whether myeloma, macroglobulinaemia, systemic amyloidosis, or related disorders have developed.

A number of epidemiological studies have explored the role of CAS by utilising medical history information, and in particular, past exposures to viral and bacterial illnesses, immunizations, allergies and autoimmune disease (Gramenzi et al, 1991; Boffetta et al, 1989; Williams et al, 1989; Cuzick and De Stavola, 1988; Cohen et al, 1987; Koepsell et al, 1987; Linet et al, 1987; Pearce et al, 1986; Gallagher et al, 1983). Based on information from the literature, including the above studies, the role of CAS in myeloma is inconclusive. This is mainly because of inconsistencies in the definition of CAS, varying quality of medical history information and the subsequent contradictory study results. Gramenzi et al (1991), found that scarlet fever, BCG vaccination and chronic bacterial disease were significant risk factors for myeloma. Risk increased with increasing number of bacterial illnesses. A nonsignificant twofold risk was observed for history of herpes zoster, pyelonephritis, tuberculosis and malaria. In contrast, two other studies showed no increase in risk for scarlet fever, tuberculosis or BCG vaccination (Cuzick

and De Stavola 1988; Koepsell et al, 1987). Linet et al (1987), observed no association between myeloma and chronic bacterial infections, while another USA based case-control study showed a trend of increased risk of myeloma with increased number of bacterial illnesses (Koepsell et al, 1987). In addition, the latter study also reported significantly elevated risks for fever blisters, contact with mononucleosis and rheumatic fever, and nonsignificant increased risks for tuberculosis, urinary tract infections and syphilis (Koepsell et al, 1987). Elevated risks for myeloma have also been reported with allergy related conditions, including hay fever, asthma, use of asthma medication and food allergies (Williams et al, 1989; Pearce et al, 1986; Gallagher et al, 1983).

With regard to autoimmune disease, no association was noted in a number of case-control studies (Gramenzi et al, 1991; Boffetta et al, 1989; Cuzick and De Stavola 1988; Cohen et al, 1987; Linet et al, 1987), while a number of other studies did show an association with rheumatoid arthritis (Eriksson, 1993; Pearce et al, 1986; Isomaki et al, 1978; Wegelius and Skrifvars, 1970; Goldenberg et al, 1969; Zbigniew et al, 1967), Sjögren's syndrome (Zawadski and Edwards, 1967) and other autoimmune diseases (Farhangi and Osserman, 1978; Magnus-Levy, 1938). Similarly an excess of diabetes was found among myeloma cases in one study (Boffetta et al, 1989), but no association was noted in two further studies (Cuzick and De Stavola 1988; Gallagher et al, 1983). As noted, the inconsistencies in the medical conditions reported by case-control studies suggest that CAS, when evaluated in this manner does not provide sufficient evidence to incriminate immune stimulation in the development of myeloma. Lewis et al (1994), are of the opinion that until a biological marker is developed that can measure lifetime

immune stimulation, it is unlikely that the relationship between CAS and myeloma can be adequately evaluated by epidemiological studies.

With regard to the racial disparity in myeloma i.e. myeloma being twice as common in blacks than whites, repeated or CAS of the immune system has been suggested as a risk factor. Lewis et al (1994), in their study of 573 cases and 2131 population-based controls, found no causal relationship between CAS and myeloma, in order to explain the higher incidence in blacks on this basis.

Viruses have also been suspected as aetiologic agents in plasma cell disorders and B cell neoplasms. Among the viruses that have been directly or indirectly associated with myeloma are Kaposi's sarcoma associated herpesvirus/Human herpesvirus 8 (KSHV/HHV-8), Human immunodeficiency virus (HIV) and Epstein-Barr virus (EBV).

KSHV is the eighth and most recently described human herpesvirus (HHV-8). It is a nonubiquitous virus first identified in Kaposi's sarcoma (KS) tissue obtained from AIDS patients (Chang et al, 1994). The virus was identified through the application of representational difference analysis to discern DNA sequences in KS that were absent from normal DNA. It is the first human member of the gamma-2 herpesviruses (genus rhadinovirus). The closest known relative of HHV-8 is the well characterized herpesvirus saimiri (HVS) of squirrel monkeys. HHV-8 has been partially sequenced recently and many regions show sequence homology at the DNA level to both EBV and HVS. The viral genome consists of linear double-stranded DNA of about 165kb in length and, like other rhadinoviruses, contains numerous open reading frames with homology to known cellular genes (Neipel et al, 1997; Russo et al, 1996). However, some of the homologous

genes encoded by HHV-8 are not shared by other rhadinoviruses or any other viruses. One of these genes encodes a structural equivalent of interleukin-6 (IL-6) with striking similarity to human and murine IL-6 (Neipel et al, 1997; Moore et al, 1996). Burger and colleagues (1998), recently found that HHV-8 IL-6 (vIL-6) is functionally active on human malignant plasma cells. This finding is of particular importance, because IL-6 has been shown to be a major growth factor for human myeloma cells (Klein et al, 1995; Klein et al, 1990).

Accumulating evidence since the first description of KSHV in 1994, leaves little doubt that this virus is the infectious cause of Kaposi's sarcoma (KS). Longitudinal studies provide the strongest evidence that KSHV is causal for KS in that both polymerase chain reaction (PCR)-based detection of KSHV DNA in peripheral blood and tumour tissues as well as antibody seroconversion studies show that KSHV infection occurs before the development of the tumour and is highly predictive for tumour development (Gao et al, 1996; Boshoff et al, 1995; Whitby et al, 1995). In addition to AIDS related or epidemic KS, HHV-8 DNA sequences have also been demonstrated in the classic and endemic forms of the disease from different geographical backgrounds (Ambroziak et al, 1995; Dupin et al, 1995; Huang et al, 1995; Moore et al, 1995; Su et al, 1995). It is now estimated that more than 95% of all cases of KS harbour HHV-8 DNA sequences in the tumour lesion. In KS, the cellular types infected by HHV-8 are heterogenous, and include the flat endothelial cells lining vascular spaces as well as the typical spindle cells, which are the cell types thought to represent the neoplastic elements of KS (Boshoff et al, 1995). In addition, in AIDS-related patients with KS, HHV-8 infection is also

detectable in the circulating B lymphocytes of approximately 50% of cases (Moore et al, 1996; Whitby et al, 1995). Moreover, HHV-8 infection of B lymphocytes in AIDS patients may precede KS development by upto 21 months (Moore et al, 1996).

HHV-8 DNA has also been detected in HIV-positive and HIV-negative cases of a rare form of lymphoma called body cavity-based lymphoma (BCBL) or primary effusion lymphoma (Cesarman et al, 1995). These tumours lack c-myc gene rearrangements but often although not always, contain EBV as well as HHV-8. In addition, HHV-8 has been detected in multicentric Castleman's disease, an angiolymphoproliferative lesion sometimes found in association with KS or AIDS (Soulier et al, 1995). More recently, the virus has been associated with primary amyloidosis and Waldenstrom's macroglobulinemia (Agbalika et al, 1998; Rettig et al, 1997a).

Seroepidemiological studies of HHV-8 prevalence in KS risk groups appear to confirm the notion that HHV-8 is likely to be a sexually transmitted infectious pathogen (Gao et al, 1996; Kedes et al, 1996). However, other routes of transmission have also been described, such as vertical transmission (Bourboulia et al, 1998). Serologic studies show that the prevalence of HHV-8 infection in various geographic and risk-group populations parallels the incidence of KS in these populations. For example, the rate of HIV-negative KS follows a pattern where the tumour is least common in the USA and the UK, more common in Italy and other Mediterranean countries, and most common in Central African countries. The KSHV seroprevalence among blood donors and other control populations follows this same geographic pattern, with less than 5%-10% of USA blood donors being seropositive, whereas the infection rate among persons from Kampala,

Uganda, can exceed 50% (Gao et al, 1996; Simpson et al, 1996). Similarly, several studies (Kedes et al, 1997; Kedes et al, 1996; Simpson et al, 1996), have found approximately 30% seroprevalence rates using a variety of antigens. The seroprevalence rate in the general population in South Africa is not known, but is likely to be intermediate between the low rates in the USA and UK and the high rates in East Africa. In a recent study of rural South African patients attending the Hlabisa Hospital, KwaZulu-Natal, the overall seroprevalence rate of 136 patients (including 50 patients with a sexually transmitted disease, 50 adult medical ward patients and 36 paediatric ward patients) was 34.6% (Wilkinson et al, 1999). In another local study which included patients from Chris Hani Baragwanath Hospital (where the present study was undertaken), the prevalence of anti-HHV-8 antibodies was 32% (95% CI 28%-38%) in 3293 subjects with cancer (excluding Kaposi's sarcoma), while the seroprevalence rate was 83% in 51 subjects with Kaposi's sarcoma. For the 108 patients with myeloma, the seroprevalence rate was 24% for any positive signal and 1.5% for a high-intensity signal using an indirect immunofluorescence assay for the ORF 73 antigen (Sitas et al, 1999).

Kedes et al (1996), used an immunofluorescence assay which detects antibodies directed against latency associated nuclear antigens (LANA) in B cells latently infected with HHV-8. They examined serum samples of 913 patients from different Kaposi's sarcoma risk population cohorts. Their results showed that HHV-8 is not a ubiquitous infection in the general population, which contrasts clearly with the known properties of other herpes viruses (such as EBV) in that they infect a relatively large population and remain in a latent state throughout the life of the host.

Recently, Rettig et al (1997b), published findings suggesting the involvement of the Kaposi's sarcoma-associated herpesvirus (KSHV) in the development of myeloma. KSHV was found in the bone marrow dendritic cells of 15/15 myeloma patients but not in malignant plasma cells (0/10) or bone marrow dendritic cells from normal individuals or patients with other malignancies (0/16). In addition, the virus was detected in 2 out of 8 patients with MGUS. Viral interleukin-6 (vIL-6), a homologue to human IL-6 has recently been identified in the KSHV genome (Moore et al, 1996). Also, dendritic cells have been shown to play a role in the growth and differentiation of mature B-cells and to increase (by 30- to 300- fold) the secretion of immunoglobulins G and A by B-cells (Dubois et al, 1997). Taken together, these data suggest that KSHV may play a role in the transformation of MGUS to myeloma and the propogation of fully malignant cells once myeloma has become manifest. KSHV infection of dendritic cells localizes the virus to the bone marrow microenvironment where viral genes (such as the gene encoding vIL-6) are expressed and may support the growth of a malignancy by infecting the malignant clone. In the study by Rettig et al (1997b), expression of viral IL-6 (vIL-6) was present in 3/3 myeloma stromal cell cultures. Therefore, KSHV has been proposed as a possible causative agent in the pathogenesis of myeloma.

The findings of Rettig and colleagues (1997b), are supported by two further studies in which KSHV was detected in 12/25 (48%) and 2/3 (67%) of cultured bone marrow dendritic cells (Agbalika et al, 1998; Tisdale et al, 1997). The detection rates are lower in the latter two studies. Possible explanations are that there may be differences in the sensitivity of the primers used in different studies, or that the virus may not be universally

present. Furthermore, KSHV could be under a strict T-cell mediated immune control in myeloma patients, leading to a very difficult detection by sensitive PCR (Tarte et al, 1998). Mitterer et al (1998), in a recent study were unable to detect the KSHV genome in a serum-free media of cultured dendritic cells, as opposed to the demonstration of the virus from dendritic cells in prior studies. Olsen et al (1998), provide further evidence against the involvement of the virus in myeloma. In their study, all the bone marrow biopsies (0/16) and negative controls (0/4) were vIL-6 negative.

The demonstration of the virus in leukapheresis products has been less successful. In four studies, KSHV was not detectable or was present in only a small proportion of patients, the percentage being similar to the background seroprevalence rate (Cull et al, 1998; Tarte et al, 1998; DeGreef et al, 1997; Masood et al, 1997). One study however, did confirm the presence of the virus in leukapheresis products (Vescio et al, 1997).

The detection of KSHV in core bone marrow biopsies by PCR and ISH (in situ hybridization) was shown in 17/20 (85%) of myeloma patients by Said and colleagues (Said et al, 1997). Their results were corroborated by two studies in which the virus was detected in 5/10 (50%) and 18/20 (90%) of patients respectively (Agbalika et al, 1998; Brousset et al, 1997). Two further studies, using either Southern blotting of unnested PCR amplification products or nested PCR, failed to amplify the KS_{330} sequence (ORF 26) in bone marrow biopsies from myeloma patients (Cathomas et al, 1998; Olsen et al, 1998). In another study, Dupin et al (1999), did not observe any staining for HHV-8 LNA-1 (Latent nuclear antigen-1) from bone marrow biopsies in myeloma.

With regard to bone marrow aspirates, most of the studies have yielded a negative result (Dubois et al, 1997; Parravicini et al, 1997; Rettig et al, 1997b). The poor yield in fresh bone marrow aspirates is likely to be due to the low number of stromal cells, heavy contamination by peripheral blood, presence of up to 90% uninfected neoplastic plasma cells and insensitivity of the PCR assay (Rettig et al, 1997b).

As mentioned previously, KSHV is not ubiquitous in humans (Kedes et al, 1996). Antibodies to other herpesviruses which are ubiquitous have been detected in a high proportion of patients with myeloma (Whitby et al, 1998; MacKenzie et al, 1997; Marcelin et al, 1997; Masood et al, 1997; Parravicini et al, 1997), thus arguing against the notion that myeloma patients are unable to mount an adequate immunological (antibody) response. KSHV may be detected in the serum by PCR or by latent or lytic serologic assays. In a blinded study comparing different latent and lytic KSHV serologic assays, the latent immunofluorescent assay was the most sensitive and specific (Rabkin et al, 1998). With the exception of one study (Alsina et al, 1997), at least nine other studies did not show serological evidence of KSHV in the serum of patients with myeloma (Agbalika et al, 1998; Cathomas et al, 1998; Cook et al, 1997; Hyjek et al, 1997; MacKenzie et al, 1997; Marcelin et al, 1997; Masood et al, 1997; Parravicini et al, 1997; Gao et al, 1996). A possible explanation for the failure to detect KSHV antibodies is that myeloma patients could be infected with a variant of KSHV that can escape the immune system or that encodes for antigens not recognised by the available immunological assays (Moore, 1998).

Presently, the role of KSHV in the pathogenesis of myeloma remains controversial. Nevertheless, attempts at defining the exact link between KSHV and myeloma are of particular relevance and importance. Implications include the use of appropriate therapeutic strategies such as antiviral agents and vaccines in the management of patients with myeloma, as well as providing a clearer understanding of oncogenesis. Feasibility studies evaluating vaccination strategies (using idiotype protein pulsed dendritic cells) are being done (Reichardt et al, 1996). This may need to be reviewed in the light of the finding of KSHV in the dendritic cells of myeloma patients. It is hoped that further studies will clarify the precise role of KSHV in myeloma.

Infection with human immunodeficiency virus (HIV) is generally considered to be primarily a disorder of cell mediated immunity (CMI). Although the primary target for HIV is the CD_4 T lymphocyte (other cells such as macrophages may also be infected by the virus), B-cell disorders are not uncommon in HIV-positive patients (Orenstein et al, 1997; Lane et al, 1983). Hypergammaglobulinaemia is common, usually manifesting as a polyclonal increase. Monoclonal or oligoclonal paraproteins have also been reported in the serum of HIV-positive patients (Konrad et al, 1993; Turbat-Herrera et al, 1993; Crapper et al, 1987; Sala et al, 1986; Heriot et al, 1985). Konrad et al (1993), described an HIV-positive patient with myeloma whose IgG-κ paraprotein specifically recognized the HIV p24 *gag* antigen, while prior reports have shown myeloma proteins to be directed against a variety of infectious agents, including bacteria such as streptococcus (Potter, 1971; Seligmann et al, 1968; Mansa and Kjems, 1965). These reports suggest a possible causal role between antigenic stimulation and myeloma. Turbat-Herrera et al

(1993), evaluated the significance of the presence of serum monoclonal paraproteins in a small group of HIV-positive patients (5 of 18 patients tested revealed monoclonal spikes on serum protein electrophoresis and immunofixation), by evaluating plasmacytosis and the presence and/or absence of atypical plasma cell infiltrates and monoclonality in the bone marrow of the patients. In those patients who had a monoclonal spike, the plasma cells did not demonstrate κ or λ light chain restriction by immunohistochemical stains. The percentage of plasma cells varied from 10 to 30% and atypical plasma cells from 5 to 20%. Plasma cell aggregates were present in every case, but were variable in number and generally small. Therefore, based on this study, the paraproteins observed are likely to be a reflection of B-cell overactivation and that plasma cell hyperplasia of the bone marrow with atypical plasma cells and plasma cell aggregates, occurs in an HIV seropositive population in the absence of plasma cell neoplasia. It is important to be aware of this entity as the erroneous diagnosis of a plasma cell neoplasia can easily be made otherwise.

Deregulation of the immune system as occurs in AIDS (Acquired Immune Deficiency Syndrome), could predispose one to the development of B-cell neoplasms. Retroviruses are associated with the development of B-cell lymphoid neoplasms through mutagenicity resulting from insertion of viral genomes into the cellular DNA and as a result of the transforming ability of certain recombinant retroviruses (Payne et al, 1982). Unlike Kaposi's sarcoma and high grade B-cell non-Hodgkin's lymphoma (small, non-cleaved, non-Burkitt or Burkitt type and diffuse large B-cell lymphomas), myeloma is considered not to be increased in individuals with human immunodeficiency virus (HIV) infection

(Levine and Gill, 1987). However, a recent study by Goedert et al (1998), reports a 4.5 fold increase in myeloma among people with AIDS.

There are reports of the two diseases occurring in the same individual, whether it be coincidental or causal (Nogues et al, 1996; Nosari et al, 1996; Piras et al, 1996; Kumar et al, 1994). As myeloma is a malignancy of plasma cells, which are terminally differentiated B-lymphocytes, the possible reasons speculated for the association between non-Hodgkin's lymphoma (NHL) and HIV may apply to myeloma.

In some reports, Epstein-Barr virus (EBV) has been incriminated. Impaired T-cell surveillance in HIV infection may allow the EBV to cause an intense polyclonal B-cell expansion, in addition to the exuberant polyclonal response from HIV per se, and other virus induced factors, with eventual selection of a monoclonal, malignant, B-cell clone (Birx et al, 1986; Groopman et al, 1986; Pahwa et al, 1986; Schnittman et al, 1986; Yarchoan et al, 1986).

With regard to Burkitt's lymphoma in the setting of HIV seropositivity, the speculated potential mechanisms for lymphomagenesis are similar to that described for NHL. Ongoing B-cell proliferation induced by either HIV or EBV and chronic antigenic stimulation in the setting of immunodeficiency create an environment in which mutations in critical oncogenes such as c-myc and or tumour suppressor genes may occur, along with abnormal DNA rearrangements leading to a chromosomal abnormality (e.g. t 8;14, t 8:22, t 8;2), clonal selection and malignant transformation (i.e. the development of a monoclonal lymphoma) (Levine, 1992).

Myeloma in association with HIV infection occurs in younger patients (mean age 37 years), compared to the classical presentation in middle aged and elderly individuals. Moreover, the clinical presentation appears to be atypical and unusual (Piras et al, 1996; Schulz et al, 1996; Kumar et al, 1994). Plasmacytomas are common, as is localisation in other extramedullary sites. Non-secretory tumours with polyclonal hypergammaglobulinaemia prevail over secretory tumours, usually occurring without concomitant suppression of non-myelomatous gammaglobulins. The demonstration of EBV genomic sequences in tumour tissue of two of the three patients in one study, suggests that the virus may contribute to the pathogenesis of plasma cell neoplasia in at least some of the patients who are HIV positive (Kumar et al, 1994). A direct role for HIV in the emergence of a myeloma-associated paraprotein is also suggested by the finding of a paraprotein specifically directed against the HIV-1 p24 antigen (Konrad et al, 1993). As expected, the prognosis is worse if myeloma is associated with HIV, more particularly in those patients who have AIDS.

1.1.7 Socioeconomic Status

A number of studies have examined myeloma incidence in relation to socioeconomic status. Johnston et al (1985), in a hospital-based case-control study during the period 1976-1982, found an odds ratio of 1,6 (95% CI, 1-2,6) for the association of home ownership and myeloma incidence and a weak trend of increasing risk with occupational level. Their study demonstrated no association between myeloma and family income and education. A number of other studies looking at various parameters of socioeconomic

status (including income, educational level, occupation and social class) and myeloma incidence showed no relationship between the two (Boffetta et al, 1989; McWhorter et al, 1989; Cuzick and De Stavola 1988; Nandakumar et al, 1986; Vågerö and Persson 1986). Two further studies were suggestive of a positive association (Koessell et al, 1996; Williams and Horm, 1977). In the latter study, the risk of myeloma in both men and women was inversely associated with socioeconomic status, being higher in individuals of lower socioeconomic status.

The present overall impression is that the association of socioeconomic status with myeloma that was reported in earlier studies of mortality (Lenhard et al, 1987; Velez et al, 1982; Blattner et al, 1981; Hoover et al, 1975; MacMahon, 1966) may possibly have been the result of better access to sensitive diagnostic methods and adequate medical treatment by individuals of higher socioeconomic status rather than a true positive relationship between an increased risk of myeloma and lower socioeconomic status.

1.1.8 Aetiological Heterogeneity of M-component subtypes

There are differences in the normal physiological function of immunoglobulin isotypes. It may therefore be conceivable that environmental factors that trigger or contribute to malignant transformation of the various classes of M-components might also differ. IgD myeloma (although very rare), has been shown to occur at an earlier age of onset (65% of the cases were under 60 years) and with a higher male to female ratio (75% of the cases were male) (Jancelewicz et al, 1975). It has a poorer prognosis compared to the commoner isotypes (Kyle and Greipp, 1988). Because IgG, IgA and light chain myeloma

are the dominant isotypes, more attention has been paid to them in epidemiological studies. In a case-control study conducted by Herrinton et al (1993), to determine whether risk factors differ with respect to the individual immunoglobulin types of myeloma, the % M-components were IgG (56%), IgA (22%), light chain disease (16,7%), IgM (2,8%), IgD (0,2%), IgE (0,3%) and two components (2,2%). Individuals having the first three common isotypes of myeloma did not differ with respect to distribution of race or age, but a slightly higher proportion of light chain cases were women. IgA myeloma was associated modestly with a history of exposure to diagnostic X-rays. Detailed analysis of the IgG and IgA subtypes provided little evidence that they differ significantly with respect to prior immune stimulation or employment in several specific occupations.

Prior studies showed modest differences between IgG, IgA and light chain myeloma with respect to immune-stimulating conditions (Williams et al, 1989), and a higher proportion of IgA myeloma cases having antecedent gastrointestinal and respiratory diseases (Schafer and Miller, 1979). These associations were not found in the more recent study by Herrinton et al (1993).

1.2 IMMUNOPHENOTYPE AND DNA CONTENT IN MYELOMA

Myeloma is a monoclonal B-cell disorder characterized by the accumulation of a population of malignant plasma cells (terminally differentiated B-lymphocytes). The normal ontogeny of B-cells has been reviewed by MacLennan et al (1995), Van Riet and Van Camp (1993), and Uckun (1990). Briefly, the following points are pertinent to this discussion. The development of B-cells into Ig secreting plasma cells occurs in two successive phases; the first phase concerns the differentiation of a primitive stem cell into mature IgM/IgD expressing B-cells, is antigen-independent and occurs in the bone marrow. The second phase, the development of resting B-cells into plasma cells and memory B-cells, is antigen-dependent as well as T-cell dependent, and occurs in secondary lymphoid organs such as the lymph nodes, tonsils, spleen and Peyer's patches. B-cells are derived from a single lymphoid stem cell that differentiates from a pluripotent stem cell. The earliest identifiable cell type that is committed to the B lineage is the pro-B cell. The pro-B cell undergoes rearrangement of its immunoglobulin heavy chain (IgH) genes and matures into a pre-B cell. This stage is characterised by the cytoplasmic expression of IgH μ chains (the heavy chain of IgM). The pre-B cell subsequently rearranges the light chain genes (κ then λ). This enables the cell to express surface IgM (sIgM) and to become an immature B-cell. B lymphocytes with sIgM positivity leave the bone marrow by migrating through the sinusoidal capillaries and enter the circulation. Soon after entering the circulation, surface IgD (sIgD) expression occurs. These sIgM and sIgD expressing B-cells, also called virgin B-cells, are arrested in the Go phase of the cell cycle. Circulating B-cells enter the secondary lymphoid organs through high

endothelial venules. Without antigen contact, they re-enter the circulation and have a lifespan of only a few weeks. However, if presented with antigen, they become activated and develop into either a short-lived plasma cell or a memory B-cell. The primary formed antibody secreting cells are always localised in the extrafollicular area of the lymph node or splenic red pulp. Memory B-cells can enter the primary follicles where they are confronted with antigen presenting dendritic cells resulting in the development of a secondary immune response. At this stage, primary follicles change into secondary follicles containing germinal centres. Subsequent re-exposure to antigen allows the memory B-cells to differentiate into centroblasts within the germinal centre. At this stage, Ig isotype switching and somatic mutation of the immunoglobulin genes occur. The genetic switch mechanism allows IgM bearing precursors to synthesize IgG, IgA and IgE. Centroblasts do not express sIg. Centroblasts develop into centrocytes that re-express sIg. Centrocytes with high affinity sIg differentiate into B-lymphoblasts. These cells may either develop into memory B-cells or, alternatively, home to the bone marrow or other areas of secondary lymphoid tissue and differentiate into plasma cells (MacLennan et al, 1995; Van Riet and Van Camp, 1993; Uckun, 1990).

The maturation and differentiation of lymphoid precursor cells into mature cells is accompanied by characteristic changes in cell surface antigen expression. The analysis of these antigens was used initially to understand the maturational pathways and stages of normal differentiation, and further to assist in the diagnosis and classification of haematological malignancies. At present, more attention is being paid to either their functional or pathogenetic role, as well as to their prognostic significance.

Flow cytometry has largely contributed to the study of the immunophenotypic characteristics of both the malignant clone and other immunoregulatory cells that may play a role in the control of myeloma, providing a higher specificity and sensitivity to these investigations (San Miguel et al, 1995). In addition, flow cytometry has facilitated the analysis of DNA content (ploidy).

In this section, the immunophenotypic characteristics of plasma cells and DNA content of plasma cells will be briefly reviewed. Two other allied aspects, viz., the immunophenotypic changes in the immunoregulatory cells and the cell cycle distribution will not be specifically discussed.

1.2.1 Immunophenotypic characteristics of Plasma cells

The myeloma plasma cells found in the bone marrow are the malignant counterparts to the normal plasma cells. Like malignant plasma cells, normal plasma cells appear to be found predominantly in the bone marrow, reflecting the affinity of plasma cells for the bone marrow microenvironment. Studies of the immunophenotype of the malignant plasma cell preceded those of the normal plasma cell, as increased number of these cells are usually detected in the bone marrow of patients with myeloma.

The origins of the malignant plasma cell remain uncertain. The neoplastic bone marrow plasma cells are end stage cells with only limited self-renewal and proliferative capacity in vivo (Bartl et al, 1995). This has led to the hypothesis that the tumour clone also includes more immature B-cells that constitute a proliferating pool of precursor cells (Pileri and Tarocco, 1974). The malignant clone appears to arise from a pre-switch B

cell (most probably a memory B-cell) which has undergone somatic hypermutation of Ig genes, indicating that myeloma originates from a germinal centre lymph node B-cell (Epstein et al, 1995; MacLennan et al, 1995; Van Riet et al, 1995). Myelomatous plasma cells can therefore be regarded as post germinal centre cells that have undergone antigen selection, isotype switch recombination and somatic hypermutation of their Ig genes.

In addition, circulating B-cells that share the clonal Ig rearrangement, can be found in the peripheral blood of myeloma patients (Bakkus et al, 1994; Billadeau et al, 1992). These circulating clonal B-cells appear to differ in their expression of adhesion molecules from normal B-cells. The clonal B-lymphocytes may be responsible for the disease dissemination in myeloma and may represent the proliferative compartment in myeloma (Pilarski and Jensen, 1992).

Variable and discrepant results with regard to the immunophenotype of plasma cells have been reported in the literature (Ruiz-Argüelles and San Miguel, 1994; Harada et al, 1993; San Miguel et al, 1991; Grogan et al, 1989; San Miguel et al, 1986; Durie and Grogan, 1985; Anderson et al, 1983). This may be related to methodological problems and lack of correct standardization of the techniques used. Monoclonal antibodies (MoAbs) of the same cluster of differentiation (CD), (e.g. CD10 antibodies) may detect different epitopes and therefore give discrepant reactivities (San Miguel et al 1991). The fluorochrome used for conjugation of the MoAb (fluorescein isothyocyanate, phycoerythrin, phycoerythrin/cyanine-5 or PerCP) may modify the threshold for positivity by its effects on the intensity of the signal and thus the number of reactive cells detected. Furthemore, multistaining techniques with plasma cell related markers for an

accurate and specific assessment of the expression of different antigens on plasma cells was infrequently used in the past . The detection system used for the visualisation of the immunological reaction is also relevant and important. Compared to flourescence microscopy and APAAP (immuno-alkaline phosphatase) techniques in the study of plasma cells, flow cytometry has a higher sensitivity. In addition, a higher number of cells are analyzed, it facilitates the performance of double and triple marker analysis, it permits the quantitative assessment of flourescence intensity and, since plasma cells display a singular light scatter pattern (FSC/SSC), they can easily be recognised.

1.2.1.1 Antigenic characteristics of myelomatous plasma cells

1.2.1.1.1 B-cell related markers

Early studies on myeloma patients showed that they usually displayed very low reactivity or even lacked the expression of classically considered pan-B-lymphoid-associated markers, such as sIg, CD19, CD20, CD22 and CD24, as well as HLA class II antigens (Ruiz-Argüelles and San Miguel, 1994; Omedè et al, 1993; San Miguel et al, 1991; Tominaga et al, 1989; San Miguel et al, 1986), despite the fact that they are considered to belong to the B-cell lineage. FMC7 antigen which appears to be restricted to mature B- cells was also absent from myelomatous plasma cells (San Miguel et al, 1986). As a consequence of this, plasma cells were initially considered antigenically 'mute', with the presence of cIg as the only immunological hallmark of myeloma. However, later studies conducted in larger series of patients, in which more sensitive techniques were applied, showed that plasma cells express B-cell antigens in approximately 15-40% of patients

(San Miguel et al, 1995; Pellat-Deceunynck et al, 1994; San Miguel et al, 1991). Using appropriate dual labellings for the identification of plasma cells, only 22% of myeloma patients are positive for CD20 and 36% for HLA-DR. Moreover, the expression of these antigens is restricted to a minority of plasma cells (<40%) (San Miguel et al, 1991). CD19 was found to be positive in 35% of patients (Pellat-Deceunynck et al, 1994), while in another study, it was negative in most myelomatous plasma cells (Harada et al, 1993). Interestingly, Omedè et al (1993), reported the presence of sIg in one third of patients (classically myeloma patients are considered to be negative for sIg). They demonstrated that the presence of sIg in myelomatous plasma cells is related to an 'early' plasma cell phenotype, lymphoplasmacytoid morphological characteristics and a poor patient outcome. CD10 (CALLA), a B-cell marker which is frequently observed in B lymphoid malignancies such as childhood acute lymphoblastic leukaemia (ALL), has been noted with a variable frequency of 10-60% in myelomatous plasma cells (Ruiz-Argüelles and San Miguel, 1994; San Miguel et al, 1991; Epstein et al, 1990; Warburton et al, 1989). This variability is probably due to differences in the epitope identified by the different MoAbs included within the CD10 cluster of differentiation (San Miguel et al, 1991; Haralambindon et al, 1987). More importanly, the presence of this antigen in myeloma has led to the postulate that pre-B cells could be the target cell in the pathogenesis of the disease. However, CD10 is also present in both normal germinal centre cells and plasma cells (Terstappen et al, 1990; Warburton et al, 1989). The influence of CD10 expression in the evolution of myeloma is controversial. While Durie and Grogan (1985), have related it to very aggressive disease (4 patients with CALLA positive myeloma had a

median survival of 6 months, compared to the CALLA negative group with a median survival of 56 months), Epstein et al (1990), have suggested that CD10+ patients display a more favourable clinical course. CD20+ myclomas display a more aggressive course, especially if their plasma cells simultaneously co-express the CD10 antigen (San Miguel et al, 1991).

1.2.1.1.2 Plasma cell-associated markers

Plasma cells are characterized by the expression of cytoplasmic immunoglobulin. However, surface bound immunoglobulin and specific B-cell antigens such as CD19, CD20 and HLA-DR, are lost from the cell during B-cell development. The absence of specific surface markers for plasma cells prompted several groups to immunise mice with plasma cells to produce MoAbs against plasma cell antigens. However, most of the MoAbs obtained were non-specific since they also identified other haemopoietic cells. Some examples of these MoAbs include R1-3 (Gonchoroff et al, 1986), 62B1, 8A (Tazzari et al, 1987), PCA-1 and PCA-2 (Anderson et al, 1983). An exception is the MoAb B-B_4, which recognises syndecan-1 and has been shown to be a highly sensitive and specific reagent to detect benign and malignant plasma cells (Wijdenes et al, 1996; Pellat-Deceunynck et al, 1994), although it is negative in some cases of myeloma.

CD38 is an antigen that is widely distributed in the haemopoietic system and therefore not entirely suitable for plasma cell identification (as it is not plasma cell specific). However, the intensity at which it is expressed on plasma cells at flow cytometry, is clearly higher than that observed on any other cell type. This strong ('bright') expression

for CD38 (CD38 +++) has converted it into a 'specific' plasma cell marker ideal for double staining studies in which plasma cells must be identified (Orfão et al, 1994; Harada et al, 1993).

With regard to the CD9 antigen, discrepant results are noted, depending on the MoAb used. MoAbs Bu16 and BA2 gave negative results (Jackson et al, 1988), while other CD9 MoAbs such as FMC8 and FMC56 were positive in up to 60% of myeloma patients (San Miguel et al, 1986). Differences either in the binding affinity or in the epitope recognised may explain these discrepancies.

1.2.1.1.3 Adhesion molecules and natural-killer associated antigens

Myeloma precursor cells generated in secondary lymphoid tissue circulate in the peripheral blood and migrate specifically to the bone marrow where they come into close contact with stromal cells and receive appropriate signals to proliferate and differentiate into plasma cells. Homing of plasma cells to the bone marrow as well as homotypic and heterotypic interactions between the B-cell clone and microenvironmental stromal cells are mediated by a large series of adhesion molecules, which also regulate cytokine secretion, primarily IL-6. Altered expression of several adhesion molecules on myeloma cells favours the haematogenous spread of the disease and the development of plasma cell leukaemia.

Myeloma plasma cells express a variety of adhesion molecules as well as natural-killer (NK) cell subsets. NK cells are defined as effector cells that perform spontaneous cytotoxic activity against autologous and allogeneic target cells (Richards and Scott,

1992; Robertson and Ritz, 1990). From the phenotypic point of view, cells displaying NK activity express a heterogeneous array of surface antigens, including CD56, CD16, CD57, CD2, CD7 and CD8 (Richards and Scott, 1992; Rees, 1990; Robertson and Ritz,1990). One of the primary roles of NK cells is their contribution to the early defence against tumours.

Cellular adhesion molecules that have been studied in myeloma include those that belong to the following groups, viz., i) integrins, ii) immunoglobulin gene superfamily, iii) selectins and iv) cell surface proteoglycans: syndecans and CD44.

The CD56 molecule is an isoform of the Neural Cell Adhesion Molecule (N-CAM), which mediates homotypic adhesion (Lanier and Hemperley, 1995). It is expressed on neural cells and has been detected in patients with malignancies such as small cell lung cancer, Wilm's tumour, Ewing's sarcoma and neuro-ectodermal tumours (Carbone et al, 1991; Figarella-Branger et al, 1990; Roth et al, 1988; Lipinski et al, 1987), as well as in myelomatous plasma cells of up to two thirds or more of myeloma patients (Orfão et al, 1994; Harada et al, 1993; Van Camp et al, 1990). In addition, myeloma cells express a wide range of cell surface adhesion molecules, such as CD49d (VLA-4), CD49e (VLA-5), CD58 (LFA-3), CD11a/CD18 (LFA-1), CD44 (H-CAM), CD54 (ICAM-1), Syndecan-1, $\alpha 4\beta 7$, CD51/CD61 (vitronectin receptor), CD102 (ICAM-2) and CD50 (ICAM-3) (Helfrich et al, 1997; Pellat-Deceunynck et al, 1994; Ruiz-Argüelles and San Miguel, 1994; Harada et al, 1993; Kawano et al, 1993). These molecules may contribute to the anchoring of plasma cells to the bone marrow via specific adherence to the marrow extracellular matrix proteins and/or to bone marrow stromal cells. This would

explain why VLA-5 negative myeloma cells circulate and are detected in the peripheral blood (Kawano et al, 1995), as well as the correlation between the loss of CD56 antigen and extramedullary spreading of malignant plasma cells in myeloma patients (Pellat-Deceunynck et al, 1994). Lack of CD56 expression has also been noted with light chain disease and associated with a shorter survival (Van Camp et al, 1990).

From the pathogenetic point of view, it has been observed that myelomatous plasma cell adhesion triggers IL-6 production by stromal cells, which promotes cell growth (Kawano et al, 1995). Pellat-Deceunynck et al (1995), found that loss of LFA-1 expression correlated with higher proliferative capacity in myeloma. The population of cells that has lost LFA-1 appears the same as the VLA-5 negative cells: such cells have an immature phenotype with plasmablastic features, actively proliferate, display responsiveness to IL-6 and a tendency to extramedullary spread, while VLA-5 positive cells have a very low proliferative activity and do not respond to IL-6, although they secrete higher amounts of paraprotein (Kawano et al, 1993). VLA-5 negative immature plasma cells may therefore be correlated with an adverse prognosis (Kawano et al, 1993).

Adhesion molecule expression in MGUS has not been extensively studied. Plasma cells in MGUS express VLA-4, VLA-5, ICAM-1 and CD44 (Harada et al, 1993; Barker et al, 1992; Leo et al, 1992; Van Riet et al, 1991). In general, the lower level of NCAM (CD56)-positive plasma cells in MGUS may be of relevance, since NCAM-mediated adhesion to bone cells has now been shown to be a factor in the induction of cytokines important in the survival of plasma cells in the marrow compartment (Barille et al, 1995).

Patients with plasma cell leukaemia, in contrast to myeloma, express multiple β1 integrins and do not express high levels of NCAM or LFA-3 (Pellat-Deceunynck et al, 1995; Barker et al, 1992; Jensen et al, 1991). Loss of these adhesion molecules may play a role in the dissemination of the disease into the circulation by reducing cell-cell interaction and may also allow the cells to escape immune surveillance.

A comparison of adhesion molecule expression between normal and malignant plasma cells is depicted in Table 1.4.

Table 1.4 Comparison of adhesion molecule expression between normal and malignant plasma cells

RECEPTOR	NORMAL PLASMA CELL	MALIGNANT PLASMA CELL
β1 integrin (CD29)	++	=
VLA-4 (CD49d)	++	=
VLA-5 (CD49e)	++	+
LFA-1 (CD11a)	++	+
LFA-3 (CD58)	-	+++
NCAM (CD56)	-	++
SYNDECAN-1	++	=
CD44	++	=
RHAMM	-	++
MPC-1	-	++

++ receptor expressed; - receptor not expressed

+++ increased expression; + decreased expression; = no change in expression

Adapted from Helfrich et al, 1997

1.2.1.1.4 Stem cell-related markers: CD34 and c-kit

The stem cell marker, CD34 antigen, is not expressed in plasma cells or any of the possible clonal cells of myeloma identified by using the heavy chain variable region gene sequence as a marker of clonality (Vescio et al, 1994). This finding has therapeutic implications for reducing tumour contamination in autografts by using positive selection of CD34 cells. In contrast, plasma cells have been shown to proliferate in response to the c-kit ligand (stem cell factor receptor, SCF-R) and expression of the CD117 (c-kit) antigen has been observed in myeloma patients (San Miguel et al, 1995; Lemoli et al, 1994).

1.2.1.1.5 Myeloid-associated markers

The expression of granulomonocytic (myeloid) related antigens (CD13, CD33, CD15 and CD14) has been reported in 6-65% of myeloma patients (Ruiz-Argüelles and San Miguel, 1994; Grogan et al, 1989). Myeloid markers appear to occur more frequently in patients with immature morphology and the co-expression of two or more myelomonocytic related antigens appears to confer a poor outcome (San Miguel et al, 1991; Grogan et al, 1989). Other myeloid-associated antigens, such as the erythroid marker glycophorin A and the megakaryocytic marker CD41 (glycoprotein II_B/III_A), have also been identified in myelomatous plasma cells, although its frequency is not yet well established (Epstein et al, 1990).

1.2.1.1.6 Other markers

T cell associated markers, such as CD2 and CD4, have been detected in the malignant plasma cells of some myeloma patients (Grogan et al, 1989). CD45 reactivity has been reported to be associated with immature plasma cells (Hata et al, 1994), although the expression of the antigen has not been extensively studied (Huelin et al, 1988). CD40 and CD28 are important molecules in the B and T cell lymphocyte activation pathways respectively. In the presence of interleukin 4 (IL-4) and IL-10, CD40 promotes both B cell growth and differentiation (Bancherau et al, 1991). Also, IL-4 downregulates the expression of CD20 through a protein kinase C-independent pathway that can be reversed by the anti-CD40 MoAb (Dancescu et al, 1992). Malignant cells from most myeloma patients (70%), as well as from patients with MGUS and Waldenstrom's macroglobulinemia, express the CD40 antigen (Pellat-Deceunynck et al, 1994; Tong et al, 1994).

The normal process of T-cell activation requires both an antigen-specific signal delivered through the T-cell receptor co. iplex, and a second co-stimulatory signal delivered by CD28 binding to its ligand on antigen presenting cells. This co-stimulatory signal potentiates and sustains the proliferation of activated T cells by synergizing with T-cell receptor mediated signals at the transcriptional and post-transcriptional level (Linsley and Ledbetter, 1993). Moreover, in the absence of this second signal, antigen-exposed T cells become anergic (Harding et al, 1992). CD28 recepto. 's expressed constitutively on the majority of CD4+ T cells and approximately half of CD8+ T cells (Hara et al, 1985; Hansen et al, 1980), however, CD28 has also been found on normal plasma cells,

plasmacytomas and myeloma cells (Kozbor et al, 1987). The ligands for CD28 are B7.1 and B7.2. CD28 stimulates T cell proliferation via the two counter-receptors, B7.1 and B7.2, expressed by the antigen presenting cells (Schwartz, 1992). In a study of 33 myeloma patients the expression of B7 and CD28 was examined by flow cytometry (Kornbluth, 1995). Myeloma plasma cells from half of the patients tested express CD28 but not B7. The other half are CD28- and B7-. These findings may provide an explanation for the T cell anergy seen in myeloma patients, including the inability to generate anti-tumour T cell responses, since T cell activation requires, together with an antigen specific signal, a co-stimulatory signal delivered by CD28 binding to B7 (Kornbluth et al, 1995). CD28 expression on myeloma cells is regarded to be an adverse feature, since it has only been detected in patients with accelerated disease (Pellat-Deceunynck et al, 1994).

In general, myelomatous plasma cells display a heterogenous phenotype, not only between different patients, but also within each patient, consistent with the fact that the neoplastic clone is able to undergo a certain degree of differentiation. In addition, plasma cells generally lack surface B cell-associated antigens and frequently show reactivity for non-lineage restricted markers. The B-B_4 and CD38+++ are the two best markers for identifying plasma cells which is crucial for the correct assessment of other antigens by multiple-staining procedures.

Although the expression of, or lack of expession of individual antigens has prognostic implications, upon being analyzed together with other relevant prognostic factors of the disease, the antigenic characteristics of plasma cells did not show independent prognostic

value (San Miguel et al, 1995). Additionally, loss of surface antigens may not only alter adhesive capacity, but perhaps also allow the neoplastic cell to escape from immunosurveillance (Clayberger, 1987).

1.2.1.2 Are normal plasma cells phenotypically different from myelomatous plasma cells?

Using multiparametric flow cytometry and plasma cell purification, Terstappen et al (1990), performed studies on normal bone marrow and showed that normal plasma cells display an extensive antigenic heterogeneity with a range of markers similar to that displayed by myelomatous plasma cells. They showed that plasma cells, together with the bright CD38 expression, may also express CD9, CD10, HLA-DR and CD20 antigens, as well as myeloid markers CD13 and CD33. Tominaga et al (1989), suggested that this antigenic heterogeneity may reflect a spectrum of differentiation that would range from the immature plasmablast (sIg+, CD10+, CD19+, CD20+, HLA-DR+, CD38+++) to the mature plasma cell (sIg-, CD10-, CD19-, CD20-, HLA-DR-, CD38+++). However, there are two types of markers that could help distinguish between normal and myelomatous plasma cells: i) the pan-B-cell markers (e.g. CD19) and ii) the adhesion molecules (e.g. CD56) (see Table 1.4). Using this information, Harada et al (1993), have reported that all normal plasma cells from various tissues are CD19+CD56-, whereas most bone marrow myeloma cells display the opposite pattern, i.e. CD19-CD56+. In MGUS, both CD19+CD56- and CD19-CD56+ plasma cells were found. However, Pellat-Deceunynck et al (1994), have shown that CD56 is expressed on a subpopulation

of normal plasma cells, although the level of expression is lower than that observed in myelomatous cells. Moreover, the CD19 antigen was present in the malignant plasma cells (CD38+++ or B-B_4) of one third of myeloma patients. According to these results, the differences established by Harada et al (1993), are not so clear cut, and the only phenotypic features that would discriminate normal from malignant plasma cells would be the lack of CD19 in the presence of strong positivity for CD56 (CD19-CD56++); this phenotype is never present in normal bone marrow plasma cells. In such a case, the antigenic combination could be of value for the investigation of minimal residual disease in myeloma. A point to bear in mind is that plasma cells become CD56- when associated with extramedullary spread. Most of the other adhesion molecules may also be present in normal plasma cells, but their intensity of expression changes in myelomatous plasma cells. Thus, malignancy is characterized by increased expression of CD56 and a lower reactivity for CD11a, while CD44 and CD49e remain unchanged (Pellat-Deceunynck et al, 1995). CD28 expression may also help in this distinction, since it has been reported to be negative in normal plasma cells and positive in aggressive myeloma. Nevertheless, it should be emphasized that during the early plateau phase, CD28 has been reported to be absent from the surface of myelomatous plasma cells (Pellat-Deceunynck et al, 1994). With disease progression, loss of Syndecan-1, VLA-5, CD56 and MPC-1 as well as acquisition of CD11b facilitate the haematogenous spread and the development of plasma cell leukaemia.

1.2.2 DNA content (ploidy) of plasma cells

Cytogenetic information is limited in myeloma, as there is often difficulty in obtaining analyzable metaphases, owing to the low proliferative activity of plasma cells. Accordingly, other approaches that allow the detection of DNA abnormalities in myelomatous plasma cells have been used. One such approach is the measurement of plasma cell DNA content using flow cytomety. Firstly, it allows the analysis of interphase cells and, secondly, this type of measurement provides rapid and objective information on the existence or absence of clonal abnormalities in the DNA cell content, i.e. DNA aneuploidy, which are closely related to cytogenetic characteristics. In addition, it allows for the study of the cell cycle distribution of plasma cells, which largely reflects the tumour cell kinetics.

Cell DNA content measurements by flow cytometry have been performed on a wide range of haematological malignancies including myeloma. A careful analysis of the results obtained in patients with myeloma shows marked variability with regard to both the frequency of aneuploidy and its clinical and prognostic significance. The reported frequency of aneuploidy ranges from 28-83%, and up to 85% in untreated patients (San Miguel et al, 1995). The reasons for these discrepancies were evaluated in a DNA Cytometry Consensus Conference held in October 1992 (Duque et al, 1993). The discrepancies as noted at the conference can be largely attributed to poorly designed studies, lack of sufficient follow-up or a significant number of patients, and technical artefacts. In this regard, there are several pitfalls that can generate erroneous results in the analysis of cell DNA content by flow cytometry, which may be related to the quality

of the sample, sample preparation techniques, reagents, data acquisition, analysis and interpretation. The use of samples either containing too few tumour cells or stored under inappropriate conditions, the lack of specific stainings for the identification of neoplastic cells and the use of sample preparation procedures by which tumour cells can be selectively lost or destroyed are some of the most relevant technical aspects that may interfere with the results obtained. In addition, adequate patient selection criteria should be used, since the DNA ploidy status of myeloma plasma cells may change during progression of the disease. Furthermore, chemotherapy may induce apoptosis and cell destruction, leading to a higher incidence of hypodiploid myeloma or false diploidy respectively.

In a large series of 156 untreated myeloma patients, aneuploidy was present in 58% of patients, with 56% hyperdiploidy and 2% hypodiploidy respectively (Garcia-Sanz et al, 1995). Table 1.5 shows the results of 14 studies in which the incidence of DNA aneuploidy was analyzed by flow cytometry (San Miguel et al, 1995). The results obtained are quite variable, with hyperdiploidy being the dominant pattern observed in 10/14 series. Hypodiploidy is uncc mon, occurring at a frequency of 0-15%. Hypodiploidy has been associated with a shorter survival and poor response to therapy (Morgan et al, 1989; Barlogie et al, 1985). A high incidence of hypodiploidy has been reported for plasma cell leukaemia (Shimazaki et al, 1988). However, hypodiploidy does not invariably portend an adverse prognosis. The report of Zandecki et al (1994), showed no association between hypodiploidy and prognosis. Moreover, the incidence of hypodiploidy is generally low, therefore the prognostic significance should be interpreted

with caution. Other studies have suggested that hyperdiploid myeloma could be associated with a worse prognosis (Bunn et al, 1982), especially when the DNA index is higher than 1.15 (Benchaib et al, 1994; Tafuri et al,1991), but the differences did not achieve statistical significance. In contrast, in the series of García-Sanz et al (1995), a significantly better outcome for DNA hyperdiploidy was detected. However, in a multivariate analysis DNA ploidy status did not show any independent prognostic value (García-Sanz et al, 1995).

Table 1.5 Incidence of DNA Hyper- and Hypodiploidy, as reported in the literature

		WHOLE SERIES				ONLY UNTREATED PATIENTS		
		HYPER-DIPLOID	HYPO-DIPLOID	DIPLOID		HYPER-DIPLOID	HYPO-DIPLOID	DIPLOID
AUTHOR	N	(%)	(%)	(%)	N	(%)	(%)	(%)
Latreille et al (1980)	50	76	2	22	14	85	0	15
Bunn et al (1982)	32	72	2	26	28	57	0	13
Latreille et al (1982)	115	80	3	17	41	-	-	-
Barlogie et al (1985)	71	76	7	17	71	76	7	17
Morgan et al (1989)	46	-	15	-	46	-	15	-
Danova et al (1990)	29	28	0	72	19	-	0	-
Tafuri et al (1991)	81	31	0	69	47	38	0	62
Tienhaara and Pelliniemi (1991)	46	54	7	39	46	54	7	39
Shimazaki et al (1988)	39	64	3	33	11	-	-	-
Ucci et al (1992)	10	50	0	50	10	50	0	50
Zandecki et al (1994)	46	58	13	24	46	58	13	24
Benchaib et al (1994)	36*	20	3	77	36*	20	3	77
De Martinis et al (1994)	9	56	11	33	-	-	-	-
Garcia-Sanz et al (1995)	156	56	2	42	156	56	2	42

* This group includes 8 cases of MGUS and 8 of smouldering myeloma.

Adapted from San Miguel et al, 1995

1.3 AIMS OF THE STUDY

Four different studies were performed and constitute the basis of this thesis. The aims of the studies are detailed below.

1.3.1 Case-control study

i) To evaluate and identify the role of suspected risk factors, such as occupational and environmental exposures, sociodemographic characteristics and chronic antigenic stimulation in the development of myeloma, and

ii) To relate the risk factors identified to the clinical presentation and prognosis of the disease.

1.3.2 HLA study

i) To determine the class I and class II HLA types in the peripheral blood of myeloma patients (compared to ethnically matched controls), and

ii) To compare and contrast the results with the existing body of literature.

1.3.3 HHV-8/KSHV study

To detect the presence of KSHV DNA sequences in bone marrow aspirates, bone marrow biopsy material and cultured bone marrow adherent cell samples of myeloma patients.

1.3.4 Flow cytometry study

i) To determine the DNA ploidy status of myeloma bone marrow plasma cells and to correlate the ploidy status with the clinical findings and prognosis where possible,

ii) To detect CD38 antigen expression on plasma cells and to further determine plasma cell co-expression with other antigens e.g. CD38-CD56, and

iii) To relate the plasma cell co-expression findings to the prognostic factors identified in the general background myeloma population (i.e. 170 patients in this study).

CHAPTER 2

2.0 PATIENTS AND METHODS

2.1 PATIENTS

The population studied consists of adult, black patients, attending the Chris Hani Baragwanath Hospital. Chris Hani Baragwanath Hospital is a public sector teaching hospital attached to the University of the Witwatersrand. It is situated 15 kilometres south-west of central Johannesburg, in Soweto. The hospital has 3300 beds and serves a community of roughly three million people. Approximately 600-1000 beds are dedicated to medical (internal medicine) admissions, with approximately 100 new medical admissions per day.

A total number of 170 consecutive, evaluable patients with myeloma, seen from January 1992 through to December 1997 were used to constitute the basis of the patient population studied. There were 101 males and 69 females. The mean age was 61.4 years (range 30-91 years). Three epidemiological aspects were investigated. In addition, the immunophenotype of bone marrow plasma cells as well as the DNA content (ploidy) was determined in a variable number of patients.

The three epidemiological aspects that were studied include i) A case-control study (130 patients (subset of the 170 patients) and 130 matched controls), ii) Determination of HLA (Human Leucocyte Antigens) - class I and II (62 patients, 57 are from the group of 170 patients and 100 ethnically matched controls) and, iii) Detection of Kaposi's sarcoma herpesvirus/human herpesvirus-8 (KSHV/HHV-8) in bone marrow cultures, bone marrow aspirates and bone marrow trephine biopsy material by PCR (polymerase

chain reaction) in a total of 27 patients (25 of whom are part of the 170 patients mentioned above). Bone marrow cell cultures were obtained and evaluated in 10/14 patients in whom the procedure was attempted. In addition, 9 bone marrow trephine biopsy samples as well as 20 bone marrow aspirates were analysed. For comparison, 19 control bone marrow aspirates obtained from patients with non-plasma cell disorders were used. These included 9 patients with acute leukaemia, 4 with non-Hodgkin's lymphoma, 3 with chronic leukaemias and 3 with immune thrombocytopenic purpura.

A bone marrow aspirate sample was submitted for flow cytometric analysis of marrow plasma cells, where possible. However, due to technical and other reasons (e.g. insufficient sample or an inadequate target population, a full analysis was not possible in all the patients. DNA ploidy analysis was performed in 103 patients, while analysis of CD38 antigen co-expression with other antigens was performed in 57 patients.

All the patients with myeloma were referred to the Haematology unit for investigation, confirmation of the diagnosis of myeloma and appropriate management of the disease, as well as long term follow-up. For the purposes of the study, the following definitions were applicable:

Diagnostic criteria for Multiple myeloma (Durie, 1986; Committee of Chronic Leukemia-Myeloma Task Force, National Cancer Institute, 1973).

Major criteria

I. Plasmacytoma on tissue biopsy

II. Bone marrow plasmacytosis of >30%

III. Monoclonal peak in serum IgG >35 g/l, IgA >20g/l

IV. Bence-Jones protein >1g/24 hours

Minor criteria

a. Bone marrow plasmacytosis 10-30%

b. Monoclonal peak in serum IgG <35g/l, IgA <20g/l

c. Lytic bone lesions

d. Suppression of normal immunoglobulins IgG <6g/l, IgA <1g/l, IgM<0,5g/l

The diagnosis of myeloma requires a minimum of one major plus one minor criterion or three minor criteria that must include a. + b., thus:

1. I + b or c or d
2. II + b or c or d
3. III + a
4. IV + a
5. a + b + c
6. a + b + d

Clinical staging of Multiple Myeloma (Durie and Salmon, 1975)

Durie and Salmon staging system

Stage I:	Criteria	Measured myeloma cell mass (cells x $10^{12}/m^2$)
	All of the following:	<0.6 (low)
	Haemoglobin value >10g/dl	
	Serum calcium value (corrected*) normal or ≤12mg/dl (3mmol/l)	

Bone X-ray, normal bone structure (scale 0), or solitary bone plasmacytoma

Low M-component production rates: IgG <50g/l, IgA <30g/dl

Urine light chain M-component on electrophoresis <4g/24hours

Stage II: Fitting neither stage I nor stage III: $0.6\text{-}1.2 \times 10^{12}/m^2$ (intermediate)

Stage III: One or more of the following: $>1.2 \times 10^{12}/m^2$ (high)

Haemoglobin value <8.5g/dl

Serum calcium value (corrected*) >12mg/dl (3mmol/l)

Advanced lytic bone lesions (scale 3)

High M-component production rates: IgG >70g/l, IgA >50g/l

Urine light chain M-component on electrophoresis >12g/24hours

Subclassification (A or B)

A = Relatively normal renal function (serum creatinine value <2mg/dl or <180μmol/l)

B = Abnormal renal function (serum creatinine value ≥2mg/dl or >180μmol/l)

*Corrected calcium = calcium (mg/dl) - albumin (g/dl) + 4.0

Scale of bone lesions: normal bones (0); osteoporosis (1); lytic bone lesions (2); extensive skeletal destruction and major fractures (3).

2.2 METHODS

2.2.1 Case-control study

In the case-control study, there were 130 patients (cases) with myeloma and 130 controls. All the patients were newly diagnosed and prospectively interviewed at Chris Hani Baragwanath Hospital. The hospital controls were selected (matched) to be similar in age (± 5 years), gender and ethnic group to the cases. The controls had diseases representative of the general population (examples include hypertension, diabetes mellitus, tuberculosis, pneumonia, ischaemic heart disease and chronic obstructive airways disease). Patients with haematopoietic malignancies were excluded.

A detailed structured questionnaire (see appendix A) was used to obtain information on a broad range of risk factors. Briefly, the information addressed the demographic characteristics, geographical history (residential and work), socioeconomic factors (education, occupational rank, income, home ownership, type of dwelling etc.), occupational history (all jobs ever held, duration of exposure etc.) as well as a number of specific exposures e.g. farming and agriculture, benzene, lead etc., exposure to drugs, toxins, chemicals and radiation, past and present medical and surgical illnesses (including chronic infections and allergic disorders), immunization, smoking habits, alcohol consumption etc., in both the cases and controls. The questionnaire was administered to all subjects in a face to face (direct or in-person) interview, after obtaining informed consent. The vast majority of the interviews (231/260 - 88.8%) were conducted by myself. The remainder of the interviews (29/260 - 11.2%) were conducted by trained interviewers. Where the information was incomplete or additional information was

necessary, this was obtained from a close relative of the subject. A translator was used when the interviewer could not fully understand the language of the interviewee. The statistical analysis was performed by a qualified statistician, using an SSPS windows software program. The analysis entailed the use of the odds ratio and χ^2 (chi-square) for the characteristics of interest. Survival was estimated by using the Kaplan-Meier survival analysis method, as described by Norusis, 1994.

2.2.2 HLA study

HLA-A, B and C typing was done on peripheral blood samples, according to the standard NIH microlymphocytotoxicity technique (Terasaki et al, 1974), modified to use 0.5 microlitre quantities of serum. The 180 antisera used to determine these specificities consisted of our own, as well as those obtained by national exchange with other laboratories and those obtained commercially (Biotest, Behring). For the class II antigens (DR and DQ), both the lymphocytotoxicity technique and immunofluorescence using immunobeads to isolate B-cells was used. Each individual was tested for the HLA-A, B, C, DR and DQ antigens depicted in Tables 3.32 to 3.36. The procedure was performed at the Immunology Department, South African Institute for Medical Research (SAIMR), Johannesburg.

Gene frequencies (gf) were calculated from the antigen frequency (af) by Bernstein's formula: $gf = 1 - \sqrt{1-af}$. The frequency of the 'blank' gene(s) was obtained by subtracting the sum total of defined gene frequencies from one. The gene frequencies in the different population groups were compared by the chi-square test. Haplotype

frequencies were calculated from the formula developed by Mattiuz et al (1970), using 2x2 tables of phenotype frequencies, and their significance evaluated by the chi-square test. Genetic distances, based on differences in gene frequencies were calculated by the method of Edwards and Cavalli-Sforza (1972).

2.2.3 KSHV/HHV-8 study

2.2.3.1 DNA analysis

DNA from myeloma bone marrow aspirates and bone marrow biopsy samples was extracted using a heat alkali extraction method. The method involved removing the bone marrow from the slide with distilled water, spinning it down briefly in a microfuge and resuspending the pellet in 200µl 0,05M NaOH. The resuspended pellet was left at 100°C for 10 minutes and then neutralised by the addition of 25µl 1 M Tris-HCL.

For the adherent cell cultures, a mononuclear cell layer was prepared from the fresh marrow samples using the Hypaque-Ficoll density gradient separation method. The resultant mononuclear cells were cultured as previously described (Gartner and Kaplan, 1980). A 3-5 x 10^6/ml density mononuclear cell sample was transferred into a 25cm^2 tissue flask containing Iscove's modified Dulbecco's medium (GIBCO, USA) with 10% fetal calf serum and incubated at 37°C for 6 hours. The non-adherent cells were rinsed with the medium and the remaining adherent cells were further cultured in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum, 200mM L-glutamate, 100U/ml penicillin and 100µg/ml streptomycin. The growth medium was changed weekly until a confluent adherent monolayer developed in the flask. The

monolayer was washed three times with phosphate buffered saline and the adherent stromal cells were harvested with 0,5% trypsin in phosphate buffered saline. DNA from the adherent cells was extracted using the heat alkali method. The cultured cells were spun down and the pellet was extracted in the same way as the bone marrow samples. Five microlitres of DNA from both the cultured cells and the bone marrow aspirate and biopsy samples was used for PCR. The integrity of the extracted DNA was confirmed by amplifying a region of the human β-globin gene (Gγ). PCR amplification of the $KS330_{233}$ sequence of KSHV was carried out on all DNA samples using a nested PCR assay. The two outer primers KS4 and KS5 were used in a first round of PCR amplification using the following cycling conditions: denaturation at 94°C for 45s, annealing at 60°C for 30s, extension at 72°C for 45s, repeated 30 times. A 0.5μl aliquot of the first round product was amplified using 5pmol of the second round PCR primers KS1 and KS2. The cycling conditions were as follows: denaturation at 94°C for 45s, annealing at 55°C for 30s, extension at 72°C for 45s, repeated 30 times (Chang et al, 1994).

All products were electrophoresed on a 2% agarose gel and visualised with ethidium bromide staining by UV transillumination. The 233bp product was sized according to molecular weight marker V (Boehringer Mannheim, Mannheim, Germany). Appropriate negative and positive controls were included in each PCR run. All positive results were repeated to confirm the positivity.

The bone marrow aspirates and trephines were performed at Chris Hani Baragwanath Hospital. The bone marrow cell cultures were prepared at the Haematology Department,

SAIMR, Johannesburg Hospital and the PCR analysis was done at the Haematology Department, SAIMR, Chris Hani Baragwanath Hospital.

2.2.4 Flow cytometry study

Following informed consent, patient's bone marrow aspirate samples were obtained from the superior posterior iliac crest, during diagnostic work up for suspected myeloma and submitted for flow cytometric analysis. Approximately 3mls of bone marrow was collected in a heparinised tube for DNA ploidy and immunophenotypic analysis.

2.2.4.1 Equipment

EPICS Profile II flow cytometer

Beckman centrifuge

2.2.4.2 Materials

1. Phosphate Buffered Saline (PBS)
2. PBS + 2% Fetal Calf Serum (FCS) + 0.2% Sodium Azide (NaN_3)
3. Isotonic Ammonium Chloride
4. Concentrated Propidium Iodide (PI)
5. 1% Paraformaldehyde (PFA)
6. Appropriate monoclonal antibodies and isotypic controls
7. Sterile distilled water

2.2.4.3 Methods

2.2.4.3.1 Immunophenotyping

A mononuclear cell preparation was obtained from bone marrow aspirate samples using ficoll-hypaque (density 1.077) separation. Cells were washed twice in PBS with 2% FCS. Where significant red cell contamination was present, an ammonium chloride lysis was performed (after the first wash with PBS). The white cell count was determined on a Sysmex full blood count analyser. Aliquots of 1 x 10^6 cells/ml were transferred to suitable tubes. A viability test was then performed. Optimal viability was considered to be >80%. An appropriate screening panel such as the following was used, viz.,

i) M5IgM / M1

ii) M2a / M2b

iii) CD38

iv) CD38/ CD56

v) CD38 / CD45

vi) CD38 / CD33

vii) CD38 / CD10

viii) κ /λ (Kappa / Lambda)

ix) Other, where necessary, and if sufficient sample present e.g. CD3 / 4/ 8.

Mononuclear cell isolates for cell surface staining were added to monoclonal antibody cocktails conjugated to FITC (Fluorescein isothiocyanate) or PE (Phycoerythrin) and analysed on a flow cytometer.

2.2.4.3.2 DNA ploidy

Cells were prepared for flow cytometric analysis as described in 2.2.4.3.1. 1ml of PI cocktail (0.5g of Triton x 100; 0.5g of Sodium Citrate; 25g of Propidium Iodide Powder) was added to tubes containing 10^6 cells. An appropriate sex matched blood specimen was used as a control. The test tubes were protected from light and incubated at 4°C for 30 min. and the fluorescence emitted from the PI-DNA complex quantitated after laser excitation of the fluorescent dye by the Coulter EPICS Profile II flow cytometer (Shapiro, 1988).

The coefficient of variation of the control G0/G1, S and G2/M phase peaks were <4%. Between ten thousand and fifteen thousand cells were counted for each analysis. G0/G1, S and G2/M phase populations were defined on histograms and expressed as percentages using Mod Fit LT analysis software.

The DNA index of the sample is calculated by dividing the MCN (mean channel number) of the total G0/G1 population of the patient by the control G0/G1 population on MCN. The DNA index may be normal (diploid): 0.95 - 1.05 or aneuploid, i.e. hyperdiploid: >1.10 or hypodiploid: <0.95.

An SSPS windows program was used for the statistical analysis. The characteristics of interest were compared with the use of the χ^2 (chi-square) test. Survival was estimated by using the Kaplan-Meier survival analysis method, as described by Norusis, 1994.

CHAPTER 3

3.0 RESULTS

The results will be presented under five major headings, viz., i) General background information, ii) Case-control study, iii) HLA study, iv) HHV-8 study and v) Flow cytometry study.

3.1 GENERAL BACKGROUND INFORMATION

The results of 170 consecutive patients with myeloma, seen from January 1992 through to December 1997 at the Haematology Unit, Chris Hani Baragwanath Hospital, will be presented initially. Thereafter, the results of subsets of patients in whom specific aspects of the study were performed are presented.

Of the 170 patients, there were 101 males and 69 females, with a male to female ratio of 1.45:1. The mean age was 61.4 years (range 30-91 years). The mean age for males was 61.2 years (range 30-89 years) and for females 61.7 years (range 32-91 years). The age at presentation in the different decades is shown in Table 3.1. The peak frequency was in the 6^{th} (29.4%) and 7^{th} (32.3%) decades. Only 12 patients (7.1%) were less than 40 years of age, while 9 patients (5.3%) were over the age of 80.

The major symptoms at presentation are noted in Table 3.2. The most frequent presenting feature was bone pain, occurring in 94.1% of patients. Other common symptoms were those related to anaemia (81.2%), infection (59.8%) and hypercalcaemia (38.5%).

Table 3.1 Age at presentation

AGE	NUMBER	%
30-39	12	7.1
40-49	10	5.9
50-59	50	29.4
60-69	55	32.3
70-79	34	20
80-89	8	4.7
90+	1	0.6

Table 3.2 Symptoms at presentation in 170 myeloma patients

SYMPTOMS	YES (Present)	NO (Absent)	TOTAL (Number)	%
Bone pain	159	10	169	94.1
Anaemia	138	32	170	81.2
Infection	101	68	169	59.8
Symptoms of hypercalcaemia	65	104	169	38.5
Bleeding	16	153	169	9.5
Symptoms of renal failure	29	140	169	17.2
Spinal cord compression	31	138	169	18.3
Symptoms of hyperviscosity	34	134	168	20.2

The signs at presentation are shown in Table 3.3. Anaemia was the dominant clinical sign occurring in 140/170 patients (82.4%). Anaemia was commoner in females (89.9%) compared to males (77.2%). Plasmacytomas were seen in 40.6% of individuals at presentation (see Figure 3.1). The radiological hallmark of myeloma is the presence of 'punched out' lytic bone lesions, which was present in 88.4% of the patients. The most classical site of involvement was the skull (see Figure 3.2). Vertebral compression fractures were seen in 50.3% of patients (see Figure 3.3). Pathological fractures were

less common (14.8%). The humeri and femurs were the typical sites of involvement. A pathological fracture of the left humerus is shown in Figure 3.4. Amyloid occurring in association with myeloma was an unusual finding (1.8%).

Table 3.3 Signs at presentation in 170 myeloma patients

SIGNS	YES (Present)	NO (Absent)	TOTAL (Number)	%
Lytic bone lesions	145	19	164	88.4
Pathological fractures	25	144	169	14.8
Vertebral compression	84	83	167	50.3
Plasmacytoma	69	101	170	40.6
Amyloid	3	165	168	1.8
Anaemia	140	30	170	82.4
Males Hb <13 g/dl	78	23	101	77.2
Females Hb <12 g/dl	62	7	69	89.9

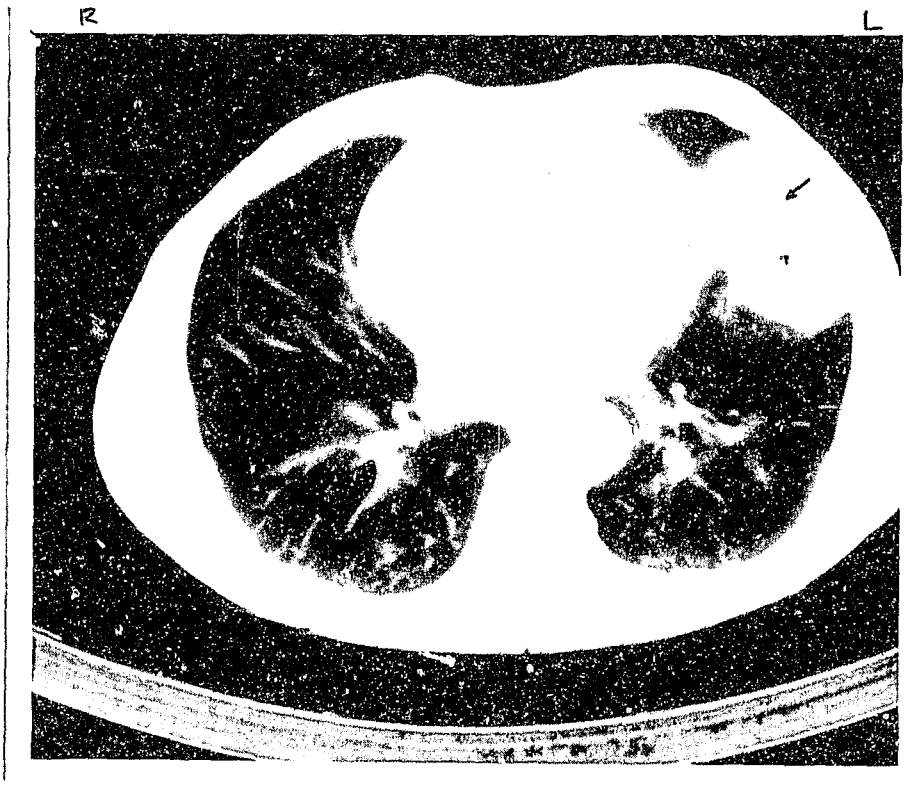


Figure 3.1 CAT Scan of chest showing a left pleural based plasmacytoma.

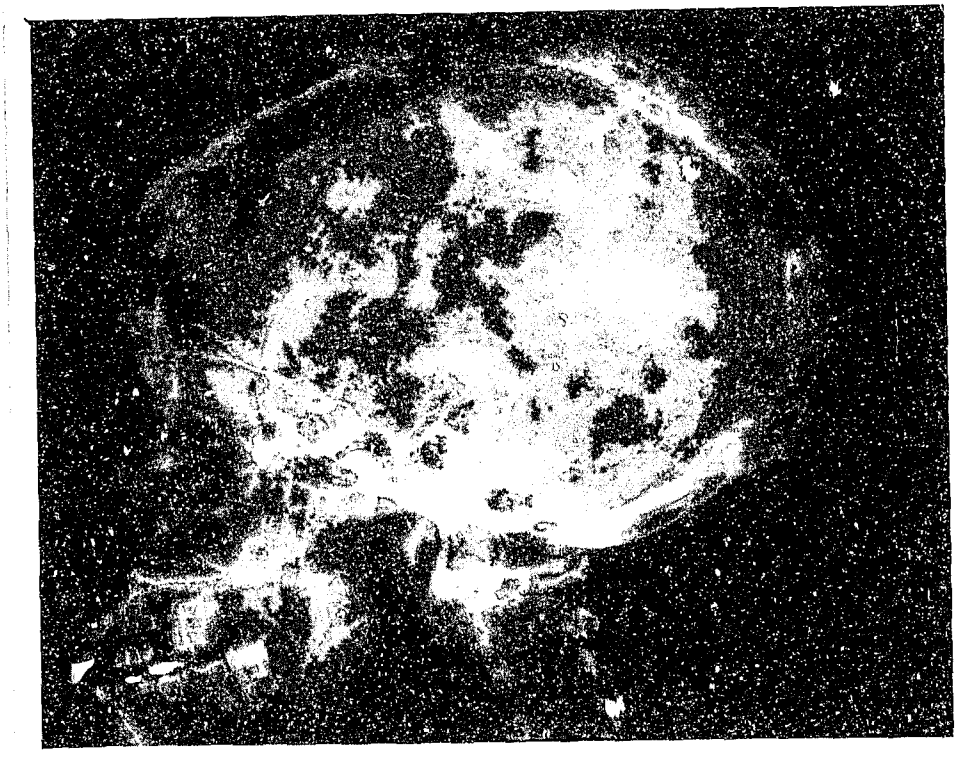


Figure 3.2 Lateral skull X-ray showing multiple punched out lytic bone lesions.



Figure 3.3 Lateral X-ray of the thoracolumbar spine showing osteopenia and vertebral compression fractures.



Figure 3.4 Pathological fracture of the left humerus. Note also the multiple lytic bone lesions present in the humerus.

Table 3.4 shows the variable performance status (PS) at presentation. A PS scale modified by the American Joint Committee on Cancer Staging and End-Results Reporting (AJC, 1978) was used. Based on this scale, five categories are defined: 0 = normal activity, 1 = symptomatic and ambulatory - cares for self, 2 = ambulatory more than 50% of time - occasionally needs assistance, 3 = ambulatory less than 50% of time - requires special nursing and medical assistance, and 4 = bedridden - may need hospitalization. Using the above scale, 56.5% of the patients had a PS of 1 or 2, while the remaining 43.5% of the patients had a poorer PS of 3 or 4. None of the patients had a PS of 0.

Table 3.4 Performance status (PS)

PS	NUMBER	%
1	51	30
2	45	26.5
3	34	20
4	40	23.5

The stage of disease at presentation is shown in Table 3.5. This is based on the Durie-Salmon staging system (see Chapter 2). The majority of patients presented with advanced stage disease (stage III - 74.6%), while early stage disease (stage I) only accounted for 12.4% of the patients.

Table 3.5 Stage of disease at presentation

STAGE	NUMBER	%
IA	20	11.8
IB	1	0.6
IIA	18	10.6
IIB	2	1.2
IIIA	104	61.1
IIIB	23	13.5
Unknown	2	1.2

The protein electrophoresis and immunoglobulin characteristics are shown in the next table (Table 3.6). A serum paraprotein on electrophoresis was noted in 83.5% of the patients, while a urine paraprotein was seen in 85.9% of patients. Kappa light chains (63%) were roughly twice as common as lambda light chains (37%). IgG myeloma was the commonest isotype (62.9%), followed by IgA myeloma (19.4%) and light chain disease (12.4%). 2 patients had IgD myeloma (1.2%). In this series, there were no patients with IgM or IgE myeloma.

Table 3.6 Protein electrophoresis and Immunoglobulin characteristics

CATEGORY	%
Paraproteinaemia	83.5
Bence Jones Proteinuria	85.9
Kappa light chains	63
Lambda light chains	37
IgG isotype	62.9
IgA isotype	19.4
IgD isotype	1.2
IgM isotype	0
IgE isotype	0
Light chain myeloma	12.4
Unknown	3.5
Nonsecretory myeloma	0.6

Table 3.7 shows the laboratory characteristics in more detail. These characteristics include both the haematological and biochemical parameters that are normally assessed in the work up of an individual with suspected myeloma. In the first column is the variable of interest together with a defined limit. The number of patients with the abnormality is reflected in the second column, with the total number of patients tested is indicated in brackets. Thus, of a total number of 170 patients, 140 (78 males and 62 females) were anaemic, as defined by a Hb of <13g/dl in males and <12g/dl in females. The percentage positive of the variable is noted in the third column. The mean value and the range of the variable are shown in the next two columns.

Table 3.7 Laboratory characteristics of 170 patients at presentation

Variable	Number	%	Mean	Range
Haemoglobin (Hb)			9.38	2.2-16.4
Total anaemic	140 (170)	82.4		
Males Hb <13 g/dl	78 (101)	77.2		
Females Hb<12 g/dl	62 (69)	89.9		
Hb <8.5 g/dl	72 (170)	42.4		
Hb <10 g/dl	106 (170)	62.4		
Urea			9.82	2-85
>8 mmol/l	46 (166)	27.7		
Creatinine			198.16	41-2620
>180 umol/l	26 (166)	15.7		
ESR			105.94	2-165
<20 mm/hr	10 (135)	7.4		
>100 mm/hr	87 (135)	64.4		
Total protein			98.44	58-169
>85 g/l	111 (166)	66.9		
>100 g/l	66 (166)	39.8		
Albumin			33.35	13-51
<35 g/l	100 (166)	60.2		
<30 g/l	55 (166)	33.1		
Globulin			65.31	20-145
>30 g/l	149 (166)	89.8		
Calcium (corrected)			2.78	2.07-6.7
>2.65 mmol/l	66 (166)	39.8		
>2.80 mmol/l	52 (166)	31.3		
>3.0 mmol/l	38 (166)	22.9		
Uric acid			0.55	0.14-1.58
>0.45 mmol/l	80 (132)	60.6		
Beta 2 microglobulin			12.90	1-593
>6 mg/l	57 (142)	40.1		

Variable	Number	%	Mean	Range
Alkaline phosphatase			120.70	36-649
<150 U/l	129 (161)	80.1		
LDH			537.59	80-4160
>450 U/l	47 (104)	45.2		
CRP			35.48	0.01-259
>12 mg/l	61 (120)	50.8		
T_4 lymphocytes			751.06	49-5480
<200 /ul	6 (107)	5.6		
<500 /ul	33 (107)	30.8		
>500 /ul	74 (107)	69.2		
T_8 lymphocytes			725.03	85-4885
<250 /ul	12 (108)	11.1		
>990 /ul	24 (108)	22.2		
T_4:T_8 ratio				
<1.0	47 (110)	42.7		
White cell count			7.81	1.2-33.3
<3.4 x 10^9/l	6 (169)	3.6		
<4.0 x 10^9/l	16 (169)	9.5		
>11.0 x 10^9/l	27 (169)	16		
Platelets			245.15	25-717
<100 x 10^9/l	17 (169)	10.1		
>400 x 10^9/l	17 (169)	10.1		
Serum paraprotein			32.47	0-96.5
>10<30 g/l	123 (165)	74.5		
>30 g/l	85 (165)	51.5		
Urine total protein			2.09	0-48.1
>150 mg/l	116 (138)	84.1		
>1000 mg/l	56 (138)	40.6		
>3000 mg/l	20 (138)	14.5		
Vitamin B12			563.99	85-2000
<100 ng/l	1 (115)	9.6		
<200 ng/l	11 (115)	0.9		

Variable	Number	%	Mean	Range
Plasma cells in BM			26.7	2-88
<10%	43 (139)	30.9		
10-30%	45 (139)	32.4		
>30%	51 (139)	36.7		

Anaemia was present in 82.4% of the patients. The mean haemoglobin (Hb) at presentation was 9.38g/dl. A Hb of <10g/dl was found in 62.4%, while a Hb of <8.5g/dl was seen in 42.4% of the patients. In contrast to the low Hb, the white cell count and platelet count was usually normal. Only 9.5% of the patients presented with a leucopenia and 10.1% with thrombocytopenia, compared to 82.4% who manifested with anaemia. The ESR was raised above 20mm/hr in the vast majority of patients (92.6%). The mean ESR was 106mm/hr. A markedly elevated ESR (>100mm/hr) was found in almost two thirds of the patients (64.4%).

An elevated total protein of >85g/l was found in 66.9% of our patients. The mean total protein was 98.44g/l. The mean globulin level was 65.31g/l, while the mean albumin level was 33.35g/l. Hypercalcaemia (>2.65mmol/l) occurred in 39.8% of patients. A calcium level above 3r. mol/l was found in 22.9%. Hyperuricaemia was common, being present in almost 2/3 (60.6%) of our patients. An elevated urea of >8mmol/l and creatinine of >180μmol/l (after rehydration) was evident in 27.7% and 15.7% respectively. Levels of beta 2 microglobulin >6mg/l were found in 40.1%, while the CRP was raised above 12mg/l in 50.8% of the patients at presentation.

In keeping with the non-osteoblastic nature of myelomatous bone disease, the alkaline phosphatase was usually normal (<150U/l in 80.1%). The LDH level was elevated in

45.2% of the patients. A T_4 lymphopenia (<500/ul) was present in 30.8% of individuals tested, while a T_8 lymphocytosis (>990/ul) was found in 22.2% of the patients. The mean paraprotein level was 32.4 g/l, with approximately half of the patients having a level above 30g/l (51.1%). The mean bone marrow plasma cell representation was 26.7%, with a range of 2-88%. More than two thirds of the patients (69.1%) had more than 10% plasma cells, while in 36.7% a level of >30% was present (see Figure 3.5).

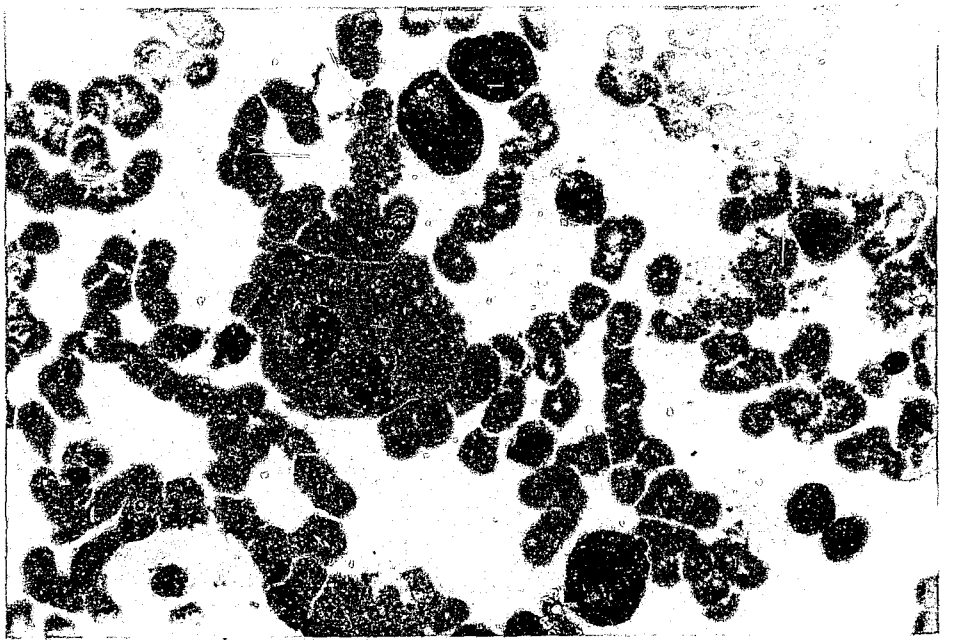


Figure 3.5 Bone marrow aspirate from a patient with myeloma showing prominent plasma cell infiltration (mostly atypical forms). Note the large binucleate plasma cell in the centre of the picture.

The prognostic significance of the variables noted in Table 3.7 were determined. Only those parameters which were of statistical significance are reflected in Table 3.8.

Table 3.8 Parameters of prognostic significance in 170 patients with myeloma at presentation

VARIABLE	MEAN SURVIVAL (months)	P VALUE
Beta 2 microglobulin		
<6 mg/l (uncorrected)	43.72	
>6 mg/l (uncorrected)	12.54	0.00005
<6 mg/l (corrected)	42.59	
>6 mg/l (corrected)	19.13	0.0153
Calcium		
<2.65 mmol/l	37.22	
>2.65 mmol/l	14.41	0.0024
<2.8 mmol/l	35.87	
>2.8 mmol/l	14.09	0.0043
Creatinine		
<180 umol/l	32.27	
>180 umol/l	4.03	0.00005
C-reactive protein (CRP)		
<12 mg/l	30.93	
>12 mg/l	21.17	0.0223
ESR		
<20 mm/hr	41.40	0.0070
>20 mm/hr	19.92	
IgG		
<50 g/l	36.35	
>50 g/l	16.49	0.0122
Platelets		
<100 x 10^9/l	5.15	0.00005
100-400 x 10^9/l	31.62	
>100 x 10^9/l	22.07	

VARIABLE	MEAN SURVIVAL (months)	P VALUE
Serum paraprotein		
<10 g/l	29.60	
>10 g/l	23.35	0.0139
Stage of disease		
IA and IB	41.55	
IIA and IIB	22.13	
IIIA	25.26	
IIIB	3.46	0.00005
Urea		
<8 mmol/l	34.77	
>8mmol/l	8.11	0.00005
Urine total protein		
>10 mg/l	22.99	
>150 mg/l	30.02	
>1000 mg/l	17.78	
>3000 mg/l	12.27	0.0021
White cell count		
<3.4 x 10^9/l	7.96	0.0446
3.4-4 x 10^9/l	14.01	
4-11 x 10^9/l	31.38	
>11 X 10^9/l	12.44	

In the second column in Table 3.8, the mean survival in months is noted with each of these parameters and the corresponding statistically significant p value is shown in the third column.

As indicated, the poor prognostic factors of statistical significance based on a univariate analysis included: a beta 2 microglobulin >6mg/l (both uncorrected and corrected for renal dysfunction, as beta 2 microglobulin is excreted by the kidneys and a falsely high level may be found in the presence of renal impairment), hypercalcaemia >2.65mmol/l,

urea >8mmol/l, creatinine >180μmol/l, CRP >12mg/l, IgG >50g/l, Platelets <100 x 10^9/l, serum paraprotein >10g/l, intermediate and advanced stage disease (especially IIIB), urine total protein >1g/l and a white cell count of <4 x 10^9/l.

The overall survival for the 170 patients was 28.37 months, with a lower median survival of 15.5 months. Figure 3.6 shows the Kaplan-Meier survival curve.

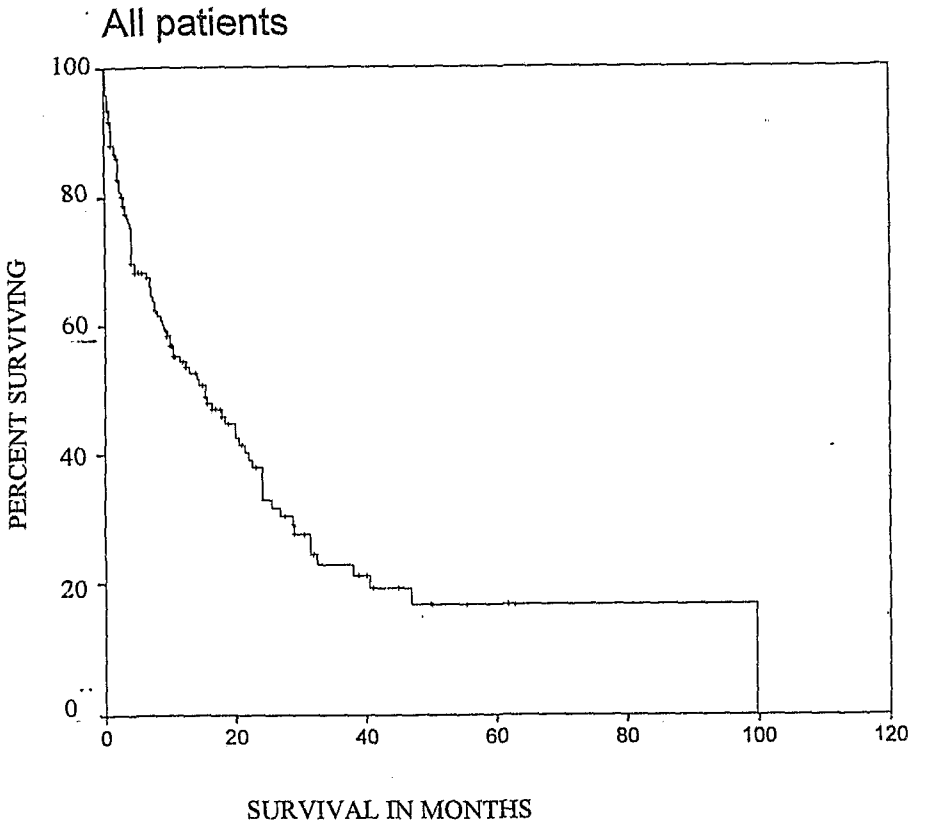


Figure 3.6 Kaplan-Meier survival curve (in months) for the 170 patients with myeloma.

3.2 CASE-CONTROL STUDY

The results of the case-control study will be presented below. 130 cases and 130 controls were interviewed prospectively, to determine risk factors for myeloma. The 130 cases are a subset of the background 170 patients discussed in 3.1. Clinical data is only presented for the cases. In both groups, 76 (58.5%) were males and 54 (41.5%) were females, with a male to female ratio of 1.45:1. The age distribution of the cases and controls is depicted in Table 3.9. The mean age at presentation was 61.5 years (range 30-91 years) for the cases and 59 years for the controls (range 35-87 years). Of the 130 patients, 8 (6.1%) were <40 years of age.

Table 3.9 Age of cases and controls

AGE	NUMBER (Cases)	%	NUMBER (Controls)	%
30-39	8	6.1	9	6.9
40-49	8	6.1	23	17.7
50-59	39	30	31	23.8
60-69	44	33.9	37	28.5
70-79	23	17.7	27	20.8
80-89	7	5.4	3	2.3
90+	1	0.8		

The dominant symptoms are shown in Table 3.10. Bone pain was the commonest symptom (93.8%), followed by symptoms of anaemia (81%) and infection (57.7%).

Table 3.10 Symptoms in 130 myeloma patients (cases) at presentation

SYMPTOMS (S)	YES (Present)	NO (Absent)	TOTAL (Number)	%
Bone pain	122	8	130	93.8
Anaemia	105	25	130	81
Infection	75	55	130	57.7
S. of hypercalcaemia	43	86	129	33.3
Bleeding	12	118	130	9.2
S. of renal failure	15	115	130	11.5
Spinal cord compression	24	106	130	18.5
S. of hyperviscosity	27	103	130	20.1

The clinical signs at presentation are shown in Table 3.11. Anaemia was found in 83% of the patients. The radiological findings were exemplified by lytic bone lesions (91.9%), vertebral compression fractures (50.1%) and less commonly pathological fractures (12.3%). Plasmacytomas were noted in 37.7% of individuals.

Table 3.11 Signs at presentation in 130 myeloma patients (cases)

SIGNS	YES (Present)	NO (Absent)	TOTAL (Number)	%
Lytic bone lesions	113	10	123	91.9
Pathological fractures	16	114	130	12.3
Vertebral compression	66	64	130	50.1
Plasmacytoma	49	81	130	37.7
Amyloid	2	128	130	1.5
Anaemia	108	22	130	83
Males Hb <13 g/dl	61	15	76	80.3
Females Hb <12 g/dl	47	7	54	87

Table 3.12 shows the PS at presentation. 39.2% of the patients had a PS of 3 or 4. None of the patients had a PS of 0.

Table 3.12 Performance status (PS)

PS	NUMBER	%
1	41	31.6
2	38	29.2
3	26	20
4	25	19.2

Table 3.13 shows the stage of disease at presentation. The majority of patients presented with advanced stage disease (stage III - 80.4%).

Table 3.13 Stage of disease at presentation

STAGE	NUMBER	%
IA	13	10
IA	0	0
IIA	16	12.3
IIB	2	1.5
IIIA	85	65.4
IIIB	12	9.3
Unknown	2	1.5

The protein electrophoresis and immunoglobulin characteristics are shown in Table 3.14. A paraproteinaemia was present in 84.6% of the patients, while a urine paraprotein was found in 85.3%. Kappa light chains (64.9%) were almost twice as common as lambda

light chains (35.1%). IgG myeloma was the commonest isotype (63.1%), followed by IgA myeloma (20%) and light chain disease (13.1%). 2 patients had IgD myeloma (1.5%), while there were no patients with IgM or IgE myeloma.

Table 3.14 Protein electrophoresis and Immunoglobulin characteristics (cases)

CATEGORY	%
Paraproteinaemia	84.6
Bence Jones proteinuria	85.3
Kappa light chains	64.9
Lambda light chains	35.1
IgG isotype	63.1
IgA isotype	20
IgD isotype	1.5
IgM isotype	0
IgE isotype	0
Light chain myeloma	13.1
Unknown	1.5
Nonsecretory myeloma	0.8

Table 3.15 shows the laboratory characteristics. The findings are similar to that noted for the general background population (170 patients). Anaemia was present in 83% of the patients at presentation. The mean Hb was 9.38g/dl. A Hb of <8.5g/dl was found in 43.1% of patients, while a Hb of <10g/dl was noted in 64.6% of individuals. Leucopenia and thrombocytopenia were less common than anaemia, occurring in 9.2% and 10% of patients respectively. An elevated total protein of >85g/l was found in 66.9% of myeloma patients. The mean total protein was 98.48g/l. The mean globulin level was 65.01g/l, while the mean albumin level was 33.68g/l. Hypercalcaemia (>2.65) was found

in 35.4% of patients. Hyperuricaemia occurred in over half the patients (58.7%). An elevated urea of >8mmol/l and creatinine of >180μmol/l (after rehydration) was evident in 20.9% and 10.9% respectively. The beta 2 microglobulin was >6mg/l in 37.6% of patients, while a CRP above 12mg/l was found in 49.5% of patients at presentation.

Table 3.15 Laboratory characteristics of myeloma patients (cases) at presentaion

Variable	Number	%	Mean	Range
Haemoglobin (Hb)			9.38	2.2-16.4
Anaemia	108 (130)	83		
Males Hb <13 g/dl	61 (76)	80.3		
Females Hb <12 g/dl	47 (54)	87		
Hb <8.5 g/dl	56 (130)	43.1		
Hb <10 g/dl	84 (130)	64.6		
Urea			8.32	2-85
>8 mmol/l	27 (129)	20.9		
Creatinine			175.15	41-2620
>180 umol/l	14 (129)	10.9		
ESR			106.13	2-165
<20 mm/hr	9 (107)	8.4		
>20 mm/hr	98 (107)	91.6		
Total protein			98.48	58-160
>85 g/l	87 (130)	66.9		
>100 g/l	53 (130)	40.8		
Albumin			33.68	18-51
<35 g/l	79 (130)	60.8		
<30 g/l	41 (130)	31.5		
Globulin			65.01	20-133
>30 g/l	116 (130)	89.2		
Serum paraprotein			33.36	0-96.5
>10<30 g/l	95 (128)	74.2		
>30 g/l	71 (128)	55.5		

Variable	Number	%	Mean	Range
IgG			44.89	2.10-142
<50 g/l	77 (128)	60.2		
>50 g/l	51 (128)	39.8		
Calcium (corrected)			2.76	2.08-6.7
>2.65 mmol/l	45 (127)	35.4		
>2.8 mmol/l	37 (127)	29.1		
>3.0 mmol/l	26 (127)	20.5		
Uric acid			0.51	0.14-1
>0.45 mmol/l	61 (104)	58.7		
Beta 2 microglobulin			12.65	1-153
>6 mg/l	44 (117)	37.6		
Alkaline phosphatase			114.37	36-475
<150 U/l	106 (128)	82.8		
LDH			551.30	149-4160
>450 U/l	35 (81)	43.2		
CRP			34.11	2.8-259
>12 mg/l	48 (97)	49.5		
T_4 lymphocytes			731.88	49-5480
<200 /ul	6 (92)	6.5		
<500 /ul	30 (92)	3.3		
>500 /ul	62 (92)	67.4		
T_8 lymphocytes			691.18	85-4885
<250 /ul	11 (93)	11.8		
>990 /ul	18 (93)	19.4		
Platelets			248.52	26-717
<100 x 10^9/l	13 (130)	10		
>400 x 10^9/l	17 (130)	13.1		
White cell count			7.86	1.2-33.3
<3.4 x 10^9/l	4 (130)	3.1		
<4.0 x 10^9/l	12 (130)	9.2		
>11 x 10^9/l	21 (130)	16.2		

Variable	Number	%	Mean	Range
Urine total protein			2.07	0-48.1
<10 mg/l	2 (111)	1.8		
>10 mg/l	109 (111)	98.2		
>150 mg/l	92 (111)	82.9		
>1000 mg/l	39 (111)	35.1		
>3000 mg/l	14 (111)	12.6		

The alkaline phosphatase level was normal in the majority of myeloma patients (82.8%), while the LDH level was elevated in 43.2% of individuals. The mean paraprotein level was 33.3 g/l, with 55.5% of the patients having a level above 30g/l. The prognostic significance of the variables noted in Table 3.15 were determined. Only those parameters which were of statistical significance are shown in Table 3.16.

Table 3.16 Parameters of prognostic significance at presentation

VARIABLE	MEAN SURVIVAL (months)	P VALUE
Beta 2 microglobulin		
<6 mg/l (uncorrected)	45.37	
>6 mg/l (uncorrected)	13.81	0.00005
<6 mg/l (corrected)	43.77	
>6 mg/l (corrected)	20.33	0.0378
Creatinine		
<180 umol/l	34.64	
>180 umol/l	4.69	0.00005
Platelets		
<100 x 10^9/l	5.83	0.00005
100-400 x 10^9/l	24.00	
>100 x 10^9/l	14.25	

VARIABLE	MEAN SURVIVAL (months)	P VALUE
ESR		
<20 mm/hr	41.40	
>20 mm/hr	21.72	0.0116
Serum paraprotein		
<10 g/l	33.12	
>10 g/l	26.09	0.0112
IgG		
<50 g/l	40.77	
>50 g/l	19.11	0.0189
Stage of disease		
IA and IB	40.29	
IIA and IIB	22.73	
IIIA	27.98	
IIIB	4.70	0.00005
Urea		
<8 mmol/l	36.24	
>8 mmol/l	10.70	0.00005
Urine total protein		
>10 mg/l	23.11	
>150 mg/l	30.72	
>1000 mg/l	21	
>3000 mg/l	13.38	0.0144

In Table 3.16, the mean survival in months is shown with each of these parameters and the corresponding statistically significant p value is also included. Poor prognostic factors of statistical significance based on a univariate analysis included: a beta 2 microglobulin level of >6mg/l (uncorrected and corrected for renal impairment), urea >8mmol/l and creatinine >180μmol/l (after rehydration), IgG >50g/l, platelets <100 x

10^9/l, serum paraprotein >10g/l, intermediate and advanced stage disease (especially stage III B), and urine total protein >1g/l.

The overall mean survival for the 130 patients was 31.95 months, with a lower median survival of 20 months. Figure 3.7 shows the Kaplan-Meier survival curve.

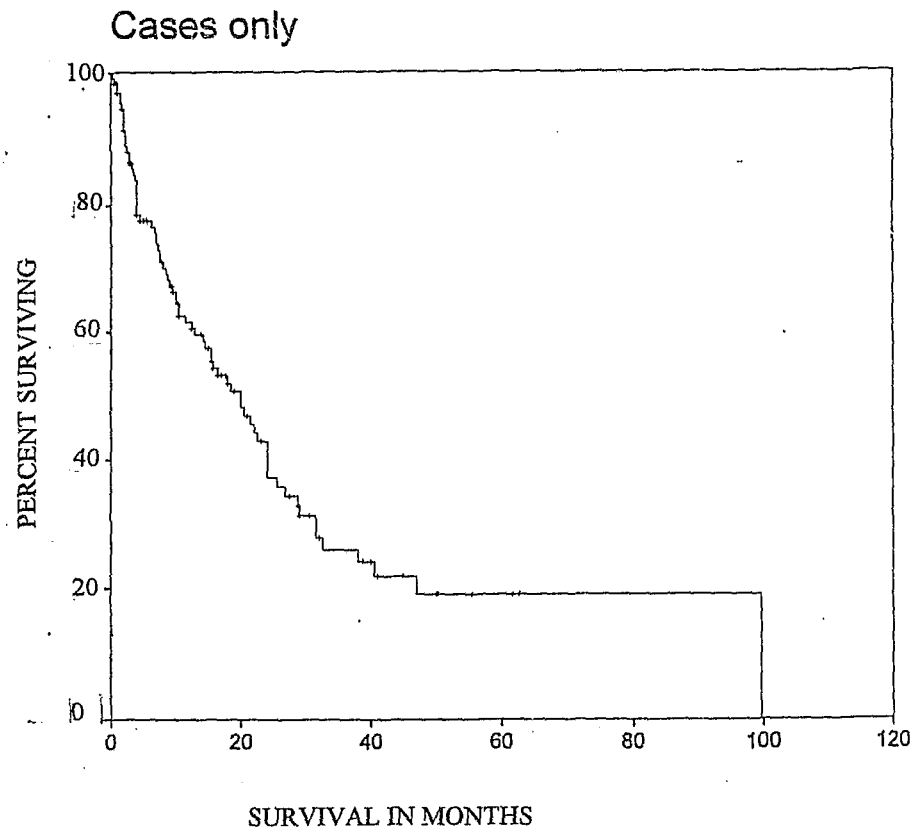


Figure 3.7 Kaplan-Meier survival curve (in months) for the 130 patients with myeloma.

The results that follow with regard to the case-control study focus on the risk factors that were looked at in the interview questionnaire (see appendix A), in both the cases and controls. Table 3.17 shows a comparison of various exposures in the cases and controls. The first two columns refer to the exposures (yes/no) in the cases and the next two to exposures in the controls. The exposures in the two groups were compared statistically. The results are reflected by the p value and odds ratio as shown in Table 3.17. The odds ratio was not adjusted for any parameters of interest. Where the odds ratio was not done, this is indicated by ND.

Table 3.17 Comparison of various exposures in cases and controls

	CASES	CASES	CONTROLS	CONTROLS	P VALUE	ODDS RATIO
	YES	NO	YES	NO		
ALCOHOL	61	69	76	54	NS	0.628
ALLERGY	8	120	4	125	NS	2.083
ANIMAL EXPOSURE	58	9	27	3	NS	0.716
APPETITE SUPPRESSANT	0	130	1	129	NS	ND
ASBESTOS	7	120	5	125	NS	1.458
ASTHMA	1	124	8	122	0.0054	0.123
BAKER/CON-FECTIONER	2	128	4	126	NS	0.492
BATTERIES	1	129	2	128	NS	0.496
BENZENE	11	119	6	124	NS	1.910
BENZO-DIAZEPINES	0	129	0	130	NS	ND

	CASES	CASES	CONTROLS	CONTROLS	P VALUE	ODDS RATIO
	YES	NO	YES	NO		
BETA BLOCKERS	1	126	5	125	NS	0.198
BUTCHER	1	129	4	126	NS	0.244
CHRONIC BRONCHITIS	0	126	1	129	NS	ND
CHRONIC OTITIS MEDIA	0	128	0	130	NS	ND
CHICKEN POX	11	108	18	110	0.0189	0.622
CATERER / COOK	22	108	17	113	NS	1.354
CHEMICALS / DYES	3	127	8	122	NS	0.360
CHRONIC ILLNESS	12	118	16	114	NS	ND
COAL TAR	1	129	1	129	NS	1
CONSTRUCT-ION	18	112	15	115	NS	1.232
CYTOTOXICS	0	129	1	129	NS	ND
DIAGNOSTIC RADIATION	77	48	109	21	0.00005	ND
DIPHENYL-HYDANTOIN	2	128	2	128	NS	ND
ELECTRICITY	9	121	12	118	NS	0.731
EXHAUST FUMES	13	54	6	24	NS	0.963
FARMING	93	37	67	63	0.0009	2.363
FIBREGLASS / M. FIBRES	2	128	0	130	NS	ND
FOOD PROCESSOR	1	129	3	127	NS	0.328

	CASES	CASES	CONTROLS	CONTROLS	P VALUE	ODDS RATIO
	YES	NO	YES	NO		
FORESTRY	2	128	0	130	NS	ND
FURNITURE / CARPENTRY	8	122	9	121	NS	0.882
HERBAL TOXINS	28	106	22	108	NS	1.065
IBUPROFEN	9	120	6	124	NS	1.550
IMMUNI-ZATION	104	11	123	3	0.0020	0.231
INSULATION	8	120	8	122	NS	1.017
LAXATIVES	68	61	55	75	NS	1.520
LEAD	7	122	6	124	NS	1.186
MALARIA	1	126	3	126	NS	0.333
MEASLES	17	101	15	112	NS	1.257
MEDICINES	1	129	3	127	NS	0.328
MINING	18	111	9	121	NS	2.180
MUMPS	7	113	6	123	0.0196	1.270
NITRO-GLYCERINE	0	130	0	130	NS	ND
PAINTER	6	124	12	118	NS	0.476
PESTICIDES	20	45	9	20	NS	ND
PHENOBARB	0	129	0	130	NS	ND
PLASTERER	2	128	7	123	NS	0.275
POWER STATION	1	129	1	129	NS	ND
RADIATION EXPOSURE (W)	0	130	3	126	NS	ND
RHEUMATOID ARTHRITIS	0	128	0	130	NS	ND
RUBBER	5	125	8	122	NS	0.610
SLE	0	128	0	130	NS	ND

	CASES	CASES	CONTROLS	CONTROLS	P VALUE	ODDS RATIO
	YES	NO	YES	NO		
SHINGLES	1	119	0	129	0.0125	ND
SMELTER	7	122	7	123	NS	1.008
SMOKING	59	71	72	58	NS	0.669
TB	4	124	17	107	0.0035	0.102
THERAPEUTIC RADIATION	0	130	2	128	NS	ND
UTI	0	128	0	130	NS	ND
WELDING	11	119	13	117	NS	0.832
WHOOPING COUGH	2	119	3	125	NS	ND
WORK ON FARM	66	64	28	100	0.00005	3.683

A statistically significant increased risk with farming (spent time at a farm, OR=2.363, p=0.0009, as well as worked on a farm, OR=3.683, p=0.00005) was noted in the myeloma cases. Higher exposures in the cases compared to controls were present with exposure to benzene, lead, asbestos, mining (underground in a coal or gold mine), fibreglass/mineral fibres, working at a building construction site, work as a caterer/cook and in the forestry industry. In all these instances, the difference between the cases and controls was not statistically significant. Exposure to alcohol and smoking were less in the cases compared to the controls. None of the cases had radiation exposure in their work environment nor did they receive therapeutic radiation prior to the diagnosis of myeloma. Furthermore, in this study the controls had statistically significantly more exposure to diagnostic radiation (usually in the form of plain X rays taken prior to this

admission) than the cases, as the controls had common diseases which generally required prior X ray investigations (109 controls versus 77 cases). A significantly higher number of controls compared to cases had TB and chicken pox, while mumps and shingles were more prevalent in the cases. The higher number of TB controls probably reflects the high prevalence of the disease in the general population.

Another aspect of the case-control study relates to the influence of socioeconomic factors. The present address, birth place and area lived most were compared in the two groups. The area within South Africa generally refers to the nine demarcated provinces which constitute the Republic. Table 3.18 shows the present address (where the person lived). Most of the controls (122-93.8%) gave a Gauteng address, with a small number presently living elsewhere. 94 cases (72.3%) gave a Gauteng address, while the remaining 27.7% came from outside Gauteng.

Table 3.18 Present address of cases and controls

Area	Cases	%	Controls	%
Northern Province	5	3.8	2	1.5
Gauteng	94	72.3	122	93.8
Mpumulanga	2	1.5	1	0.8
North West Province	7	5.4	0	0
Orange Free State	5	3.8	0	0
Kwa-Zulu Natal	9	6.9	3	2.3
Eastern Cape	3	2.3	0	0
Western Cape	0	0	0	0
Northern Cape	0.8	0	0	0
Other countries within Africa	4	3.1	2	1.5

With respect to birth place, it is noteworthy that only 33.8% of the cases and 50% of the controls were born in Gauteng (see Table 3.19 below). Kwa-Zulu Natal and Orange Free State were the next two most frequent areas of birth in both the cases and controls. Migration to the cities may account for the increase in numbers in the Gauteng area as shown in the present address. This is also borne out in Table 3.20, which shows the area lived most.

Table 3.19 Birth place (cases and controls)

Area	Cases	%	Controls	%
Northern Province	10	7.7	8	6.2
Gauteng	44	33.8	65	50
Mpumulanga	10	7.7	9	6.9
North West Province	7	5.4	9	6.9
Orange Free State	20	15.4	18	13.8
Kwa-Zulu Natal	24	18.5	13	10
Eastern Cape	2	1.5	1	0.8
Western Cape	0	0	0	0
Northern Cape	2	1.5	0	0
Other countries within Africa	11	8.5	7	5.4

In the category of area lived most, Gauteng was the most frequent 'area lived most' in both the cases and controls, but the percentage was much higher in the control group.

Table 3.20 Area lived most (cases and controls)

Area	Cases	%	Controls	%
Northern Province	5	3.8	2	1.5
Gauteng	88	67.7	118	90.8
Mpumulanga	3	2.3	2	1.5
North West Province	7	5.4	0	0
Orange Free State	6	4.6	2	1.5
Kwa-Zulu Natal	14	10.8	4	3.1
Eastern Cape	1	0.8	0	0
Western Cape	0	0	0	0
Northern Cape	1	0.8	0	0
Other countries within Africa	5	3.8	2	1.5

Table 3.21 Tribe/Language (cases and controls)

Tribe/Language	Cases	%	Controls	%
Northern Sotho (Pedi)	9	6.9	7	5.4
Tswana	25	19.2	15	11.5
Southern Sotho (Sotho)	25	19.2	32	24.6
Venda	2	1.5	0	0
Tsonga/Shangaan	4	3.1	5	3.8
Xhosa	17	13.1	15	11.5
Swazi	3	2.3	5	3.8
Zulu	43	33.1	49	37.7
Ndebele	1	0.8	0	0
Other	1	0.8	2	1.5

Table 3.21 refers to the tribe/language in both the cases and controls as shown in the appropriate columns. Zulu, Sotho, Tswana and Xhosa were the most frequently encountered tribes/languages in both groups.

Further, the categories that were studied included marital status, income, home ownership, number of persons per room (a measure of overcrowding and its relationship to disease, particularly of an infective nature), type of home, educational status and occupational rank. In Table 3.22, the difference in marital status is shown between the cases and controls. Most of the cases (72.3%) were married, compared to 50% of the controls. The other four categories (single, divorced, widow, widower) were higher in the controls.

Table 3.22 Marital Status (cases and controls)

Marital status	Cases	%	Controls	%
Married	94	72.3	65	50
Single	7	5.4	16	12.3
Divorced	4	3.1	10	7.7
Widow	19	14.6	28	21.5
Widower	6	4.6	11	8.5

Table 3.23 shows the highest nett monthly income in both the cases and controls. A similar income category (i.e. 2 and 3, which translates to an income of R301 to R1000) was found in both groups.

Table 3.23 Highest monthly income (nett income)

Income in rands	Cases	%	Controls	%
0-300	12	9.2	10	7.7
301-600	57	43.8	56	43.1
601-1000	30	23.1	30	23.1
1001-1500	18	13.8	16	12.3
1501-2000	4	3.1	12	9.2
>2000	9	6.9	6	4.6

Home ownership (i.e. a home owned or registered in the name of the individual) was very similar in both cases and controls as shown in Table 3.24.

Table 3.24 Home ownership (cases and controls)

Yes or No	Cases	%	Controls	%
Yes	87	66.9	86	66.2
No	43	33.1	44	33.8

With regard to the number of persons per room, the findings also appeared to be similar (see Table 3.25).

Table 3.25 Number of persons per room

Number	Cases	%	Controls	%
0-2	85	65.4	79	60.8
3-4	41	31.5	44	33.8
5-6	4	3.1	6	4.6
7-8	0	0	1	0.8
>8	0	0	0	0

Furthermore, there was no major difference noted in the type of home between the cases and controls (see Table 3.26 below).

Table 3.26 Type of home

Type of home	Cases	%	Controls	%
Brick house	110	84.6	109	83.8
Apartment/flat	0	0	0	0
Hostel	0	0	1	0.8
Shack	6	4.6	11	8.5
Outbuilding/backroom	0	0	1	0.8
Boarding	0	0	0	0
Other	14	10.8	8	6.2

The educational status as measured by years of formal education was similar in the two groups, although the numbers were higher in the lowest category for the cases (see Table 3.27).

Table 3.27 Educational status

Years of education	Cases	%	Controls	%
0-4	62	47.7	44	33.8
5-8	27	20.8	34	26.2
8-12	35	26.9	47	36.2
>12	6	4.6	5	3.8

Table 3.28 shows the occupational rank as a measure of social class in the cases and controls. Most of the cases and controls belonged to categories 4 and 5 (i.e. partly skilled and unskilled occupations).

Table 3.28 Occupational rank (social class)

Occupational rank	Cases	%	Controls	%
Leading professions and business jobs requiring higher education	2	1.5	0	0
Lesser professions and business jobs requiring secondary education	5	3.8	9	6.9
Skilled workers, non-manual	18	13.8	25	19.2
[illegible]tly skilled workers	55	42.3	50	38.5
Unskilled workers	48	36.9	44	33.8
Never employed	0	0	0	0
Other	48	36.9	44	1.5

The 12 most frequent occupations in the cases and controls are shown in Tables 3.29 and 3.30 respectively. A number of occupations such as a domestic worker, machine operator, clothing machinest, driver, cleaner, farm labourer and clerk were common to both groups, reflecting a similar occupational rank in both the cases and controls.

Table 3.29 Dominant occupations in the cases and controls

Occupation	Number of cases (%)	Number of controls (%)
Domestic worker	28 (21.5)	28 (21.5)
Machine operator	12 (9.2)	10 (7.7)
Farm labourer	8 (6.2)	4 (3.1)
Driver	8 (6.2)	12 (9.2)
Clothing machinest	6 (4.6)	10 (7.7)
Clerk	5 (3.8)	4 (3.1)
Miner	5 (3.8)	
Cleaner	4 (3.1)	5 (3.8)
Construction worker	4 (3.1)	
Nursing sister	3 (2.3)	
Salesman/saleslady	3 (2.3)	
Gardener	2 (1.5)	

Occupation	Number of cases (%)	Number of controls (%)
Electrician		5 (3.8)
Packer		4 (3.1)
Messenger		4 (3.1)
Teacher		4 (3.1)
Nursing assistant		3 (2.3)

3.3 HLA STUDY

Human leucocyte antigens were studied in 62 myeloma patients and 100 ethnically matched controls (except in the case of the antigen frequency analysis of the A locus, where there were 99 controls). Of the patient population, there were 39 males and 23 females. The mean age was 59.1 years, with a range of 30 - 82 years. The M:F ratio was 1.7:1.

Firstly, the results of the clinical and radiological findings as well as the laboratory characteristics of the 62 patients is presented. This is followed by a comparison of the antigen frequencies of the class I and class II loci of both the patients and controls. Thereafter, the haplotype frequencies that confer higher and lower risk of myeloma are tabulated. Finally, published associations, corresponding associations, negative associations and additional associations are presented.

Table 3.30 and Table 3.31 show the clinical and radiological characteristics and some of the laboratory features of the 62 patients with myeloma. The findings are essentially similar to that shown earlier in relation to the general background population and the 130 cases in the case-control study and do not require further explanation.

Table 3.30 Clinical and radiological features of 62 patients with myeloma

VARIABLE	%
Bone pain	93.5
Infection	48.4
Bleeding	6.5
Lytic bone lesions	87.1
Plasmacytoma	33.9
Spinal cord compression	21
Vertebral compression fractures	54.8
Pathological fractures	12.5
Stage IA	14.5
IB	0
IIA	12.9
IIB	1.6
IIIA	62.9
IIIB	8.1

The haematological and biochemical characteristics are noted in Table 3.31. The mean value, range and frequency of the variable are shown.

Table 3.31 Laboratory characteristics of 62 patients with myeloma

VARIABLE	MEAN	RANGE	%
Anaemia	9.4	2.2-15.6	82.3
Males Haemoglobin <13g/dl			79.5
Females Haemoglobin <12g/dl			87
Haemoglobin <8.5g/dl			40.3
Haemoglobin <10g/dl			64.5
White cell count	7.3	2.3-2.8	
<3.4 x 10^9/l			3.2
<4.0 x 10^9/l			8.1
>11.0 x 10^9/l			8.1
Platelets	254	47-602	
<100 x 10^9/l			8.1
<150 x 10^9/l			16.1
>400 x 10^9/l			12.9
Urea	9.41	2-85	
>8 mmol/l			16.1
Calcium	2.69	2.16-4.5	
>2.65 mmol/l			29
>2.80 mmol/l			24.2
>3.00 mmol/l			19.4
Creatinine	214.4	47-2620	
>180 umol/l			9.7
C-reactive protein	35.19	0-259	
>12 mg/l			44.9
Beta 2 - microglobulin	17.6	1-593	
>6 mg/l			37
IgG isotype			58.1
IgA isotype			19.4
Light chain myeloma			17.7
IgD isotype			1.6
Nonsecretory myeloma			1.6
Unknown			1.6

VARIABLE	MEAN	RANGE	%
Bence Jones proteinuria			90.3
Kappa light chains			67.9
Lambda light chains			32.1

The antigen frequencies (af) of the class I (A, B and C) and class II (DR and DQ) loci in the cases and controls are shown in Tables 3.32 to 3.36. The relative risk and statistical significance of the antigen in the cases when compared to controls is shown in the last two columns. Regarding the af in the class I - A locus (Table 3.32), A34 and A43 were only present in the patients and conferred a relative risk of 4.9 and 8.2 respectively. Higher af in the patients compared to the controls were also seen with A1, A2, A3, A23, A24, A28, A29, A30 and A31. However, in none of these af was the difference statistically significant.

Table 3.32 Antigen frequencies : Class I - A locus

	PTS.	PTS.	CTR.	CTR.	PATIENTS (PTS.)	CONTROLS (CTR.)	P VALUE	RELATIVE RISK
	YES	NO	YES	NO	af	af	S/NS	
A1	4	58	5	94	0.0645	0.0505	NS	1.3
A2	21	41	28	71	0.3387	0.2828	NS	1.3
A3	8	54	9	90	0.1290	0.0909	NS	1.5
A23	13	49	16	83	0.2097	0.1616	NS	1.4
A24	2	60	3	96	0.0323	0.0303	NS	1.1
A25	0	62	1	98	0.0000	0.0101	NS	0.5
A26	7	55	20	79	0.1129	0.2020	NS	0.5
A28	11	51	17	82	0.1774	0.1717	NS	1.1
A29	9	53	14	85	0.1452	0.1414	NS	1.1
A30	27	35	39	60	0.4355	0.3939	NS	1.2
A31	1	61	1	98	0.0161	0.0101	NS	1.6
A32	1	61	7	92	0.0161	0.0707	NS	0.3
A33	3	59	6	93	0.0484	0.0606	NS	0.8
A34	1	61	0	99	0.0161	0.0000	NS	4.9
A43	2	60	0	99	0.0323	0.0000	NS	8.2

The af of the class I - B locus are depicted in Table 3.33. HLA B18 and B35 were statistically significantly higher in the cases compared to controls. HLA B18 conferred a relative risk of 6.3 and HLA B35 a relative risk of 11.8. Higher af were also seen with B13, B14, B22, B27, B41, B42, B45, B60, B62 and B70.

Table 3.33 Antigen frequencies : Class I - B locus

	PTS.	PTS.	CTR.	CTR.	PATIENTS (PTS.)	CONTROLS (CTR.)	P VALUE	RELATIVE RISK
	YES	NO	YES	NO	af	af	S/NS	
B7	9	53	18	82	0.1452	0.1800	NS	0.8
B8	7	55	14	86	0.1129	0.1400	NS	0.8
B13	3	59	2	98	0.0484	0.0200	NS	2.3
B14	4	58	6	94	0.0645	0.0600	NS	1.1
B17	20	42	39	61	0.3226	0.3900	NS	0.8
B18	5	57	1	99	0.0806	0.0101	<0.05 - S	6.3
B22	1	61	0	100	0.0161	0.0000	NS	4.9
B27	1	61	1	99	0.0161	0.0100	NS	1.6
B35	3	59	0	100	0.0484	0.0000	<0.05 - S	11.8
B38	0	62	1	99	0.0000	0.0100	NS	0.5
B39	0	62	5	95	0.0000	0.0500	NS	0.1
B41	1	61	0	100	0.0161	0.0000	NS	4.9
B42	16	46	24	76	0.2581	0.2424	NS	1.1
B44	11	51	19	81	0.1774	0.1900	NS	0.9
B45	9	53	9	91	0.1452	0.0900	NS	1.7
B51	0	62	2	98	0.0000	0.0200	NS	0.3
B53	2	60	8	92	0.0323	0.0800	NS	0.5
B60	1	61	1	99	0.0161	0.0100	NS	1.6
B62	1	61	1	99	0.0161	0.0100	NS	1.6
B63	0	62	2	98	0.0000	0.0200	NS	0.3
B70	19	43	28	72	0.3065	0.2800	NS	1.1

Table 3.34 shows the af in class I - C locus. CW2, CW3 and CW6 were non-statistically significantly higher in the patients than the controls.

Table 3.34 Antigen frequencies : Class I - C locus

	PTS.	PTS.	CTR.	CTR.	PATIENTS (PTS.)	CONTROLS (CTR.)	P VALUE	RELATIVE RISK
	YES	NO	YES	NO	af	af	S/NS	
CW2	25	37	28	72	0.4032	0.2800	NS	1.7
CW3	13	49	14	86	0.2097	0.1400	NS	1.6
CW4	12	50	24	76	0.1935	0.2400	NS	0.8
CW6	20	42	31	69	0.3226	0.3100	NS	1.1
CW7	13	49	39	61	0.2097	0.3900	NS	0.4

With regard to the af in the class II - DR locus, DR1, DR3, DR8, DR10, DR11 and DR12 occurred more frequently in the patients compared to the controls. The relative risk with both DR8 and DR12 was 3.8 (see Table 3.35).

Table 3.35 Antigen frequencies : Class II - DR locus

	PTS.	PTS.	CTR.	CTR.	PATIENTS (PTS.)	CONTROLS (CTR.)	P VALUE	RELATIVE RISK
	YES	NO	YES	NO	af	af	S/NS	
DR1	7	55	11	89	0.1129	0.1100	NS	1.1
DR2	12	50	20	80	0.1935	0.2000	NS	1.0
DR3	27	35	38	62	0.4355	0.3800	NS	1.3
DR4	8	54	17	83	0.1290	0.1700	NS	0.7
DR6	19	43	32	68	0.3065	0.3200	NS	0.9
DR7	5	57	13	87	0.0806	0.1300	NS	0.6
DR8	5	57	2	98	0.0806	0.0200	NS	3.8
DR9	0	62	3	97	0.0000	0.0300	NS	0.2
DR10	4	58	3	97	0.0645	0.0300	NS	2.1
DR11	19	43	25	75	0.3065	0.2500	NS	1.3
DR12	5	57	2	98	0.0806	0.0200	NS	3.8

Table 3.36 shows the af of the class II - DQ locus. The frequency of DQ3 was higher in the patients compared to the controls.

Table 3.36 Antigen frequencies : Class II - DQ locus

	PTS.	PTS.	CTR.	CTR.	PATIENTS (PTS.)	CONTROLS (CTR.)	P VALUE	RELATIVE RISK
	YES	NO	YES	NO	af	af	S/NS	
DQ1	37	25	68	32	0.5968	0.6800	NS	0.7
DQ2	18	44	38	62	0.2903	0.3800	NS	0.7
DQ3	28	34	37	63	0.4667	0.3700	NS	1.4

In addition to the antigen frequencies, haplotype frequencies were also studied. The haplotype frequencies that conferred a higher relative risk and those that are associated with a lower risk (i.e. may be protective) are shown in Tables 3.37 and 3.38.

Table 3.37 Haplotype frequencies : Higher Risk

HAPLOTYPE	PATIENTS	CONTROLS	CHI SQUARE VALUE	PROBABILITY (* - ****)
A23 B70	0.0441	0.0197	4.2	*
A30 B42	0.0723	0.0577	5.6	**
A3 DR10	0.0115	0.0046	5.2	*
A30 DR8	0.0404	0.0036	7.1	***
A43 B22	0.0049	0.0000	30.4	****
A43 B35	0.0048	0.0000	9.2	***
B13 CW6	0.0167	0.0063	6.6	**
B70 CW3	0.0597	0.0571	7.4	***
B42 DR3	0.0749	0.0603	5.6	**
CW2 DQ3	0.1218	0.0281	6	**
DR11 DQ3	0.1226	0.1103	11.6	****

Probability grading: * = 0.05>p>0.02; ** = 0.02>p>0.01

*** = 0.01>p>0.001; **** = 0.001>p>0.000

Of the haplotype frequencies that conferred a higher risk, A43B22 and DR11DQ3 are highly significant (p=0.001>p>0.000). The two haplotypes that were protective are A2B43 and A30B70 (see Table 3.38).

Table 3.38 Haplotype frequencies : Lower Risk

HAPLOTYPE	PATIENTS	CONTROLS	CHI SQUARE VALUE	PROBABILITY (*-****)
A2 B42	0.0000	0.0065	7.4	***
A30 B70	0.0000	0.0003	12.2	****

The HLA associations that have been published in myeloma are shown in Table 3.39.

Table 3.39 Published associations

PUBLISHED ASSOCIATIONS
A3, A5
B5, B13, B18, BW65
CW2, CW5, CW6
DRW14

Of the published associations noted in Table 3.39, only HLA B18 was found in our study. HLA B35 is an additional association found in our patients (that has not been documented previously in association with myeloma). HLA B35 was not found in the control group.

3.4 HHV-8 STUDY

The patient characteristics are shown in Table 3.40. A total of 27 patients with a diagnosis of myeloma were studied. There were 16 males and 11 females. The mean age was 62.7 years (range 35-83 years). IgG immunoglobulin isotype was present in 17 patients (63%), IgA in 7 patients (26%), IgD in 1 patient (4%) and light chain disease (LCM) in the remaining two patients (7%). κ light chains were identified in 16/22 patients (73%), while λ light chains were present in 6/22 patients (22%). The majority of the patients had advanced stage disease, viz., stage 1A - 4 patients (15%), 2A - 1 patient (4%), 3A - 19 patients (70%) and 3B - 3 patients (11%).

Bone marrow aspirates were analysed in 20 patients. Only 1 patient (5%) demonstrated a positive result (see Figure 3.8). Bone marrow cell cultures were obtained in 10 patients . In 4/10 patients (40%), KSHV DNA sequences were demonstrated (see Figure 3.9). None of the bone marrow trephines (0/9) were positive. Results of PCR based detection of KSHV DNA in peripheral blood were available in 14 of the 27 patients, this being part of another study (unpublished data - results included in Table 3.40). All 14 patients had a negative result.

Table 3.40 Patient characteristics, bone marrow and serological findings

Patient Number	Age	Gender	Immunoglobulin isotype	Light chain	BM aspirate	BM cell culture	BM trephine	Serology	Stage	Name
1.	61	F	IGA	κ	--	YES	--	NEG.	3A	R.S.
2.	63	M	IGG	κ	YES	YES POS.	YES	--	3A	M.C.
3.	77	M	IGA	?	YES	--	--	--	3A	A.M.
4.	83	F	IGG	?	--	YES POS.	--	--	3A	A.V.
5.	79	M	IGG	?	YES	--	YES	--	3A	W.M.
6.	59	M	IGA	κ	--	YES	--	NEG.	1A	C.G.
7.	51	F	IGD	?	--	YES POS.	--	--	3A	E.Q.
8.	63	F	LCM	κ	--	YES	--	--	3A	M.M.
9.	61	M	IGG	?	YES	YES	YES	NEG.	1A	J.H.
10.	59	F	IGG	κ	--	YES	--	NEG.	1A	G.M.
11.	71	M	IGG	κ	YES POS.	--	YES	--	1A	D.G.
12.	35	M	IGG	κ	YES	--	YES	NEG.	3A	M.M.
13.	45	F	IGG	κ	--	YES POS.	YES	NEG.	3A	A.J.
14.	65	M	IGG	λ	YES	YES	YES	NEG.	3B	S.M.
15.	74	F	IGG	κ	YES	--	--	NEG.	3A	E.K.
16.	79	F	IGG	λ	YES	--	--	--	2A	H.M.
17.	52	M	IGG	κ	YES	--	YES	NEG.	3A	J.Y.
18.	45	M	IGA	κ	YES	--	YES	NEG.	3B	G.F.
19.	62	F	IGA	λ	YES	--	--	--	3A	M.T.
20.	76	M	IGG	κ	YES	--	--	NEG.	3A	K.T.
21.	47	M	IGG	λ	YES	--	--	--	3A	A.S.
22.	71	F	IGG	κ	YES	--	--	NEG.	3A	E.H.
23.	60	F	IGG	λ	YES	--	--	NEG.	3A	J.M.
24.	48	M	IGG	κ	YES	--	--	--	3A	G.M.
25.	75	M	IGA	κ	YES	--	--	--	3A	A.M.
26.	68	M	LCM	λ	YES	--	--	--	3B	H.M.
27.	66	M	IG. .	κ	YES	--	--	NEG.	3A	G.M.

? =unknown YES =procedure done YES POS. =positive result NEG. =negative result LCM =light chain myeloma -- =not done

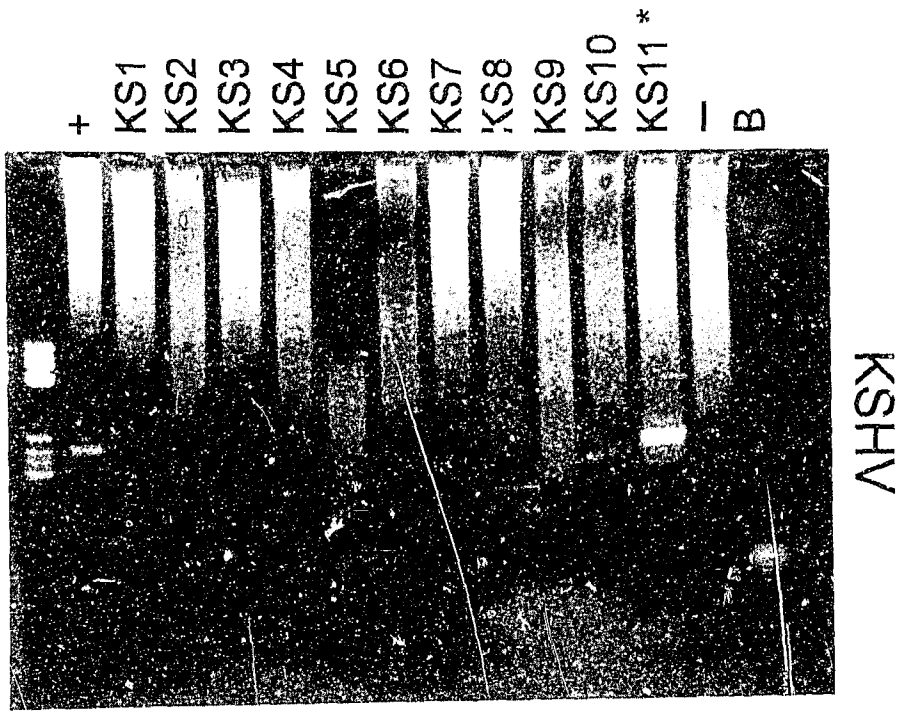


Figure 3.8 Agarose gel electrophoretogram from myeloma bone marrow aspirate DNA samples. Lanes KS1 - KS11 represent different patients. Lane KS11 shows a nested PCR amplified KSHV DNA sequence. The positive band corresponds to 233 base pairs. + denotes a positive control and - a negative control.

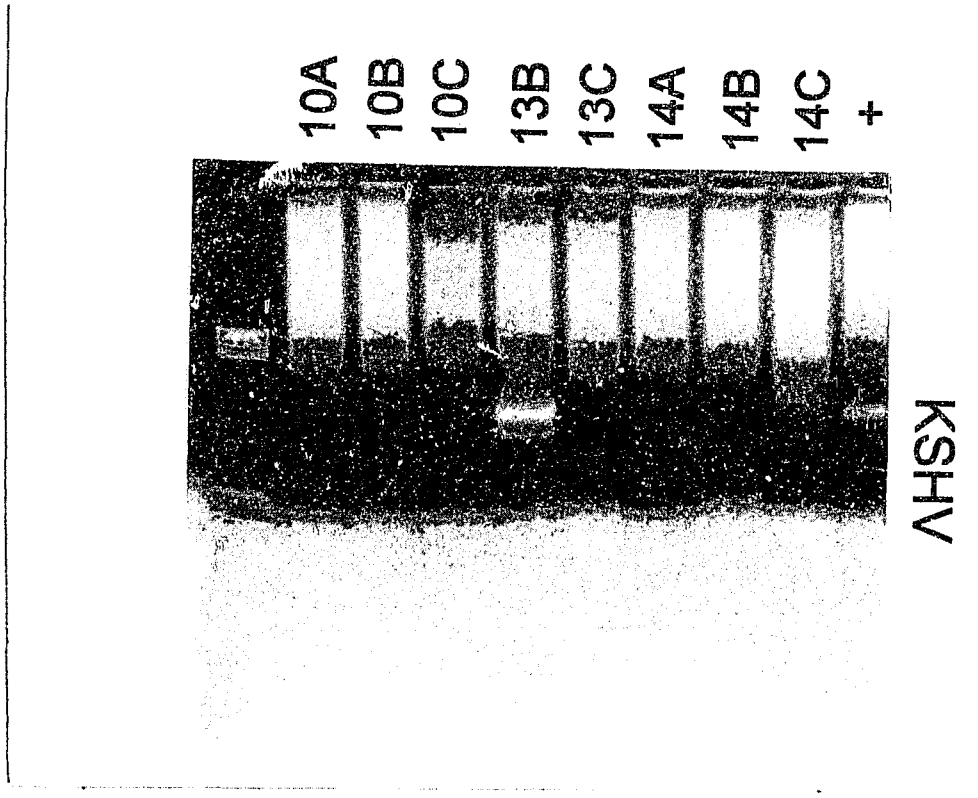


Figure 3.9 Agarose gel electrophoretogram from myeloma bone marrow cultured dendritic cell DNA samples. 10A - 14C represent different patients. Lane 13B shows a nested PCR amplified KSHV DNA sequence. The positive band corresponds to 233 base pairs.

3.5 FLOW CYTOMETRY STUDY

The results of two aspects of flow cytometry i.e. DNA ploidy and immunophenotyping are presented. DNA ploidy was performed in 103 patients. 65 patients (63.1%) had a diploid pattern, while aneuploidy was present in 38 patients (36.9%). Of these 38 patients, 2 patients (1.9%) had hypodiploidy and the remaining 36 patients (35%) had hyperdiploidy (see Figures 3.10 and 3.11). In Table 3.41, a comparison of clinical and laboratory variables are shown in both the diploid and aneuploid group. The mean age was similar in both groups. The mean bone marrow plasma cell numbers were statistically significantly higher (p=0.0005) in the aneuploid group, as were the serum paraprotein levels (p=0.0078). Regarding the other variables noted in Table 3.41, there were no significant differences between the two groups.

Table 3.41 DNA Ploidy Analysis - Comparison of the clinical and laboratory characteristics in the diploid and aneuploid groups

VARIABLE	DIPLOID	ANEUPLOID	P VALUE
Age	63.3	62.1	0.8267
% plasma cells (morphology)	20.3	35.3	0.0005
% plasma cells (flow cytometry)	11.7	23.1	0.0013
Albumin (g/l)	33.3	32.6	0.5794
Beta 2 microglobulin (mg/l)	7.9	9.0	0.1083
Calcium mmol/l	2.7	2.7	0.6817
CD10	28.8	14.0	0.0715
CD38	50.7	55.3	0.5430
CD45	79.3	83.7	0.7554
CD56	34.7	32.0	0.9782

VARIABLE	DIPLOID	ANEUPLOID	P VALUE
Creatinine (umol/l)	162.8	144.1	0.2356
CRP (mg/l)	42.1	22.8	0.2795
ESR (mm/hr)	102.3	104.4	0.8170
Globulin (g/l)	60.9	69.2	0.2200
Haemoglobin (g/dl)	10.1	9.5	0.2329
IgA (g/l)	10.7	8.8	0.1029
IgG (g/l)	41.7	57.4	0.0651
IgM (g/l)	1.0	0.8	0.3653
Platelets ($x10^9$/l)	261.4	254.6	0.6421
LDH (U/l)	520.7	639.5	0.8497
T4 lymphocytes (/ul)	859.5	593.6	0.2406
T8 lymphocytes (/ul)	898.9	661.7	0.2820
T4:T8 ratio	1.2	1.3	0.8268
Total protein (g/l)	93.8	101.9	0.1454
Serum paraprotein (g/l)	28.5	42.7	0.0078
Urea (mmol/l)	8.4	7.7	0.5543
Uric Acid (mmol/l)	0.5	0.57	0.1344
Urine total protein (mg/l)	1.6	2.5	0.3589
White cell count (x 10^9/l)	8.2	8.1	0.6201

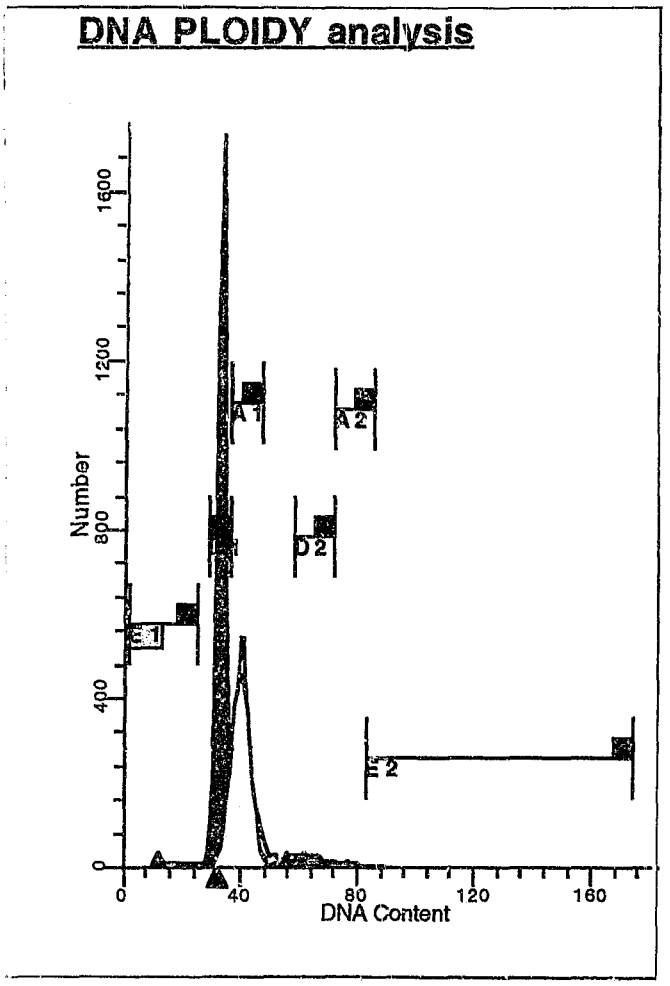
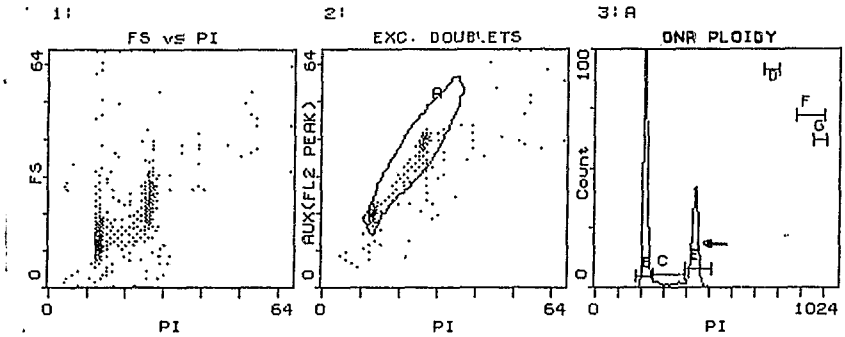


Figure 3.10 DNA ploidy histogram in a patient with myeloma. Note the aneuploid population depicted in yellow.

COULTER(R) EPICS(R) Acquisition Flow Cytometry Report

OP ID: 1

Cytosettings from protocol 1-DNA PLOIDY



Stats: Not Normalized, Listgating: Disabled

Hist	Region ID	%	Count	Mn X	Mn Y	PkPosX	PkPosY
2	A A	96.0	5283	16.3	24.3	13.0	20.0

Hist	Region ID	%	Count	Mn X	Md X	PkPosX	PkCnt	HPCV
3	B G0G1.DIP	69.6	3677	209.9	208.9	208.0	376	1.71
	C S.DIP	5.6	298	306.9	306.5	242.0	8	0.28
	D G2M.DIP	0.0	0	****	****	****	**	**
	E G0G1.ANP	24.8	1310	421.9	422.5	426.0	63	1.79
	F S.ANP	0.0	0	****	****	****	**	**
	G G2M.ANP	0.0	0	****	****	****	**	**

Hist	Region ID	%	Count	PkCnt	FPCVY	FPCVX
2	A A	96.0	5283	960	33.80	35.45

Hist	Region ID	%	Count	Min	Max	FPCVX
3	B G0G1.DIP	69.6	3677	172.0	242.0	2.96
	C S.DIP	5.6	298	242.0	380.0	13.65
	D G2M.DIP	0.0	0	710.0	770.0	**
	E G0G1.ANP	24.8	1310	380.0	488.0	3.24
	F S.ANP	0.0	0	844.0	961.0	**
	G G2M.ANP	0.0	0	918.0	974.0	**

Figure 3.11 DNA ploidy histogram in a patient with myeloma . E represents the aneuploid (tetraploid) population (indicated by arrow).

In addition to DNA ploidy, relevant plasma cell antigen co-expression analysis was performed. In Table 3.42, a summary of CD38 co-expression with CD56, CD45, CD33 and CD10 is shown.

Table 3.42 Summary of relevant plasma cell (CD38) antigen co-expression

	Number of cases YES	Number of cases NO	Significance p value
CD 38-56	45/57 (78.9)	12/57 (21.1)	0.6424
CD 38-33	15/53 (28.3)	38/53 (71.7)	0.2495
CD 38-45	25/57 (43.9)	32/57 (56.1)	0.9401
CD 38-10	5/52 (9.6)	47/52 (90.4)	0.5517

Positive expression is indicated by 'yes' and negative expression by 'no'. A higher percentage positive expression was only seen with CD56 (78.9%), while negative expression was commoner with CD33, CD45 and CD10 (see Table 3.42). Survival in the two groups (i.e. those with positive and negative antigen expression) was analyzed statistically. The p values are indicated in the last column of Table 3.42.

Prognostic factors which emerged from the background patient population analysis and the case-control study were correlated with co-expression. The findings are detailed in Tables 3.43 to 3.46. Table 3.43 shows CD38-56 antigen co-expression in relation to prognostic factors. With the exception of stage of disease, none of the other prognostic factors were significantly different in the two groups. An example of CD38-56 co-expression is shown in Figure 3.12.

Table 3.43 CD38-56 antigen co-expression in relation to prognostic factors

Variable	Yes (number)	No (number)	Significance
Beta 2 microglobulin			NS
<6mg/l	23	8	
>6mg/l	13	4	
Calcium			NS
<2.65 mmol/l	25	10	
>2.65 mmol/l	18	2	
<2.8 mmol/l	28	10	
>2.8 mmol/l	15	2	
Creatinine			NS
<180 umol/l	41	12	
>180 umol/l	4	0	
CRP			NS
<12 mg/l	16	7	
>12 mg/l	16	2	
Platelets			NS
<100 x 10^9/l	5	2	
White cell count			NS
<4.0 x 10^9/l	2	3	
Urea			NS
<8 mmol/l	36	12	
>8 mmol/l	9	0	
Urine total protein			NS
>3000 mg/l	1	2	
Stage			0.0013
I	9	2	
II	1	5	
III A	31	5	
III B	3	0	

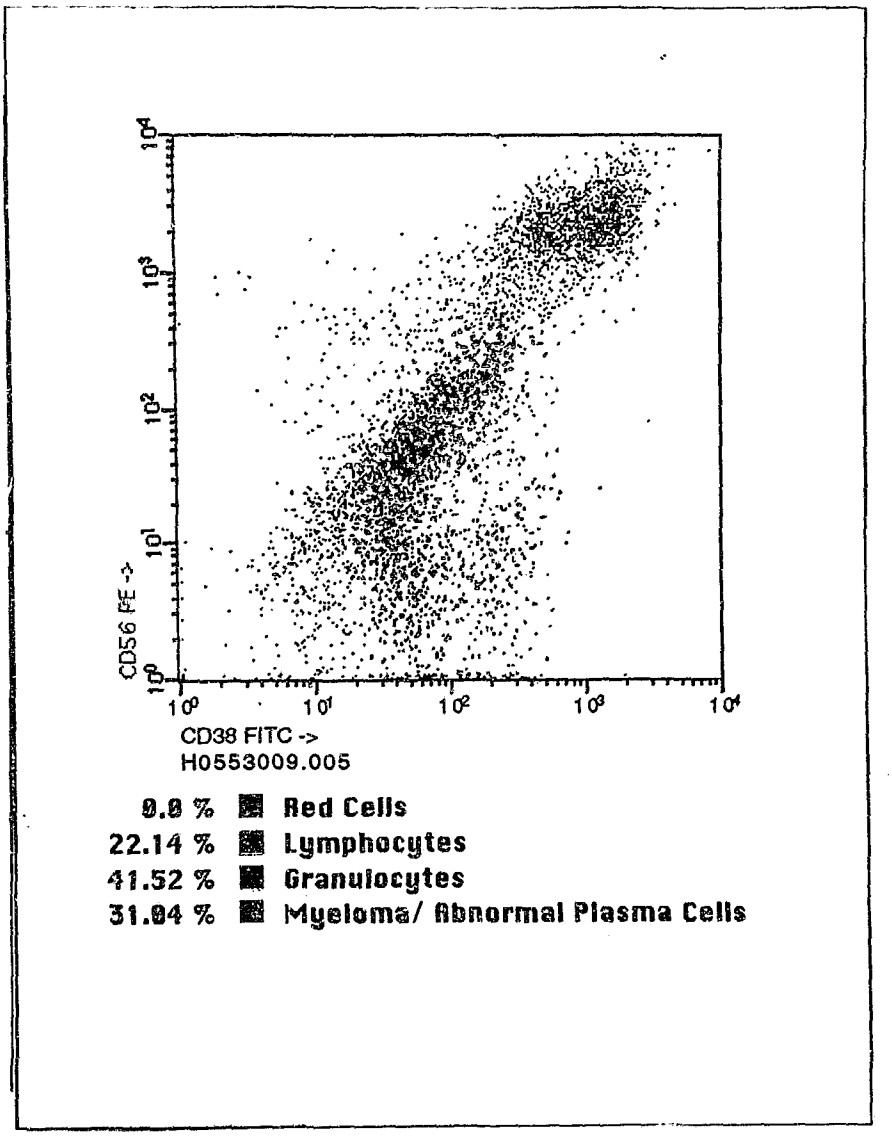


Figure 3.12 Flow cytometric analysis - Scattergram showing dual antigen CD38-56 co-expression depicted in light blue.

In Table 3.44, CD38-45 antigen co-expression is shown in relation to prognostic factors. No significant correlation was present with any of the defined prognostic factors.

Table 3.44 CD38-45 antigen co-expression in relation to prognostic factors

Variable	Yes (number)	No (number)	Significance
Beta 2 microglobulin			NS
<6mg/l	13	18	
>6mg/l	7	9	
Calcium			NS
<2.65 mmol/l	14	20	
>2.65 mmol/l	9	12	
<2.8 mmol/l	14	23	
>2.8 mmol/l	9	9	
Creatinine			NS
<180 umol/l	24	29	
>180 umol/l	1	3	
CRP			NS
<12 mg/l	7	15	
>12 mg/l	10	9	
Platelets			NS
<100 x 10^9/l	3	2	
White cell count			NS
<4.0 x 10^9/l	3	2	
Urea			NS
<8 mmol/l	23	24	
>8 mmol/l	2	8	
Urine total protein			NS
>3000 mg/l	2	2	
Stage			NS
I	2	8	
II	0	3	
III A	19	18	
III B	0	3	

In Table 3.45, CD38-33 antigen co-expression in relation to prognostic factors is shown. Positive expression was not related to any of the prognostic factors shown in Table 3.45.

Table 3.45 CD38-33 antigen co-expression in relation to prognostic factors

Variable	Yes (number)	No (number)	Significance
Beta 2 microglobulin			NS
<6mg/l	7	19	
>6mg/l	6	12	
Calcium			NS
<2.65 mmol/l	8	23	
>2.65 mmol/l	7	13	
<2.8 mmol/l	8	26	
>2.8 mmol/l	7	10	
Creatinine			NS
<180 umol/l	13	36	
>180 umol/l	2	2	
CRP			NS
<12 mg/l	9	11	
>12 mg/l	3	14	
Platelets			NS
<100 x 10^9/l	0	1	
White cell count			NS
<4.0 x 10^9/l	0	2	
Urea			NS
<8 mmol/l	11	31	
>8 mmol/l	0	11	
Urine total protein			NS
>3000 mg/l	1	2	
Stage			NS
I	2	7	
II	2	4	
III A	9	25	
III B	2	1	

Table 3.46 shows CD38-10 antigen co-expression in relation to prognostic factors. Positive expression did not correlate with any of the prognostic factors (see Table 3.46).

Table 3.46 CD38-10 antigen co-expression in relation to prognostic factors

Variable	Yes (number)	No (number)	Significance
Beta 2 microglobulin			NS
<6mg/l	3	25	
>6mg/l	1	14	
Calcium			NS
<2.65 mmol/l	3	28	
>2.65 mmol/l	2	17	
<2.8 mmol/l	3	31	
>2.8 mmol/l	2	14	
Creatinine			NS
<180 umol/l	5	43	
>180 umol/l	0	4	
CRP			NS
<12 mg/l	0	19	
>12 mg/l	3	14	
Platelets			NS
<100 x 10^9/l	0	5	
White cell count			NS
<4.0 x 10^9/l	1	4	
Urea			NS
<8 mmol/l	4	39	
>8 mmol/l	1	8	
Urine total protein			NS
>3000 mg/l	0	3	
Stage			NS
I	2	8	
II	1	5	
III A	2	30	
III B	0	3	

CHAPTER 4

4.0 DISCUSSION

Myeloma is the most common lymphoid malignancy in blacks, while non-Hodgkin's lymphoma ranks first among whites in the USA (Devesa et al, 1987). Myeloma accounts for 31% of the lymphohaematopoietic malignancies among blacks and 13% among whites (Ries et al, 1991).

Blattner et al (1979), proposed that the high rate of myeloma in blacks was not limited to the USA, but that a similar picture may be present in South African blacks. Based on the experience at Chris Hani Baragwanath (CHB) Hospital, the findings in this thesis lend further support to their findings. Indeed, myeloma is the commonest haematopoietic malignancy encountered at CHB hospital. In the period April 1987 to March 1993, of a total of 474 patients seen with haematological malignancies, 143 patients (30%) had myeloma (Patel, 1994).

The present study includes 170 patients seen over a period of 6 years (January 1992 to December 1997). The average number of new patients seen with myeloma is 28 per year. This is slightly higher than the 24 patients per year noted in the earlier study (Patel, 1994).

A total of 170 patients with myeloma were seen from January 1992 to December 1997. This constitutes the background myeloma population in this study. Initially, two aspects of the study were undertaken, viz., the case-control study and the flow cytometry study. Interviews were possible in 130 of the 170 patients. The other 40 patients were not interviewed for primarily two reasons. Firstly, a number of patients died during the initial

admission and secondly, patients who were not interviewed during the first admission did not return for follow up (lost to follow up). Similarly, flow cytometry was possible in 103 of the 170 patients. The reduced numbers were due to the following reasons: i) some patients had bone marrow aspirates performed at a peripheral hospital or by a private physician prior to being referred to our hospital and the bone marrow was not repeated by us, and ii) for technical reasons a flow cytometric analysis was not possible - insufficient or grossly haemodilute specimen, and in some instances there was a dry tap. The HLA study was commenced in 1995, by which time a number of the original patients had died and some were lost to follow up. Sixty two patients had HLA studies performed (57 of whom were part of the 170 patients).

Important information regarding the role of KSHV emerged towards the latter part of this epidemiological study. It was deemed necessary to include a brief analysis of this possible association prior to concluding this study. Thus, the numbers represented in this aspect of the study are small (25 of the 27 patients are from the background 170 patients).

Despite having recognised that myeloma is the leading cause of haematopoietic malignancies at CHB hospital, figures on the incidence rates for the whole South African population appear to be relatively low. These rates are misleading and differ from the figures quoted by Blattner et al (1979). Referral and diagnostic bias may account for some of the differences. The National Cancer Registry provides information for the whole population (including the rural and urban areas), while the study of Blattner et al (1979), was conducted in Johannesburg (at Johannesburg and Baragwanath hospitals),

where there is greater awareness of the disease and specialised diagnostic tests are more readily available. Moreover, in general, there is underreporting of myeloma cases to the National Cancer Registry, as the reporting was traditionally based only on 'histological' evidence of the disease. This erroneous method of reporting myeloma cases has subsequently been corrected (at least at Chris Hani Baragwanath hospital). The bone marrow aspirate and trephine are not always diagnostic of myeloma, and the clinical, radiological and biochemical features must be taken into account, before a diagnosis of myeloma is established or excluded. A few patients may not have had a bone marrow aspirate and trephine performed, yet have myeloma on the basis of their skeletal and biochemical findings. Therefore, myeloma should not be diagnosed or excluded on histological grounds only.

It is noteworthy that there has been a slight increase in the official myeloma incidence rates in South Africa for 1990 and 1991 (1,79/100 000 males and 1,33/100 000 females), compared to the rates for 1987 (1,5/100 000 males, 1,0/100 000 females) (Sitas et al, 1996; Cancer Registry of South Africa, 1987). With regard to blacks, the incidence rates have increased in both males and females (1987 - 1,3/100 000 males, 1,0/100 000 females; 1990 & 1991 - 1,86/100 000 males, 1,44/100 000 females). Also, the 1990 & 1991 rates are higher in black males and females compared to their white counterparts. This is in contrast to the lower rates in blacks noted in 1987. Improvements in the socioeconomic status of blacks may partly explain this difference, with greater accessibility to treatment centres and availability of diagnostic and confirmatory tests, thus improving the diagnostic yield and reducing the degree of underreporting. The

number of myeloma cases reported to the National Cancer Registry have steadily increased from 1986 to 1991, viz., 1986-99; 1987-141; 1988-158; 1989-156; 1990-199; 1991-200 (Sitas et al, 1996). These increases reflect more than just a corresponding increase in the population over this time period.

Myeloma is characteristically a disease of middle and old age. The incidence of the disease increases with age and reaches a peak during the seventh decade of life. The median age at diagnosis is approximately 65 years. In the USA, the median age at diagnosis for myeloma is slightly higher (69 for men and 71 for women), compared to the median age of diagnosis for all cancers (68 for men and 66 for women respectively) (Ries et al, 1991). In the Western world, fewer than 2% of the patients are less than 40 years of age at diagnosis (Hewell and Alexanian, 1976; Kyle, 1975). Myeloma case series from Africa have suggested a younger median age at diagnosis (five to fifteen years younger) than in the Western world, probably reflecting a younger age structure of the population (Patel et al, 1992; Jacobson 1991; Mukiibi and Kyobe, 1988; Mukiibi and Mkwananzi, 1987). These studies also report a male excess, which is consistent with the gender difference (male predominance) reported in other countries (Mukiibi and Mkwananzi, 1987; Cuzick et al, 1983; Kyle, 1975).

4.1 GENERAL (BACKGROUND) POPULATION

The initial review will focus briefly on the 170 patients, who constitute the background patient population. Following this initial review, more specific aspects of the study will be discussed. Of the 170 patients, there were 101(59.4%) males and 69(40.6%) females,

with a male to female ratio of 1.45:1. The mean age at presentation was 61.4 years (range 30-91 years). The peak age frequency was in the 6th (29.4%) and 7th (32.3%) decades. The median age in our patients is intermediate between the figures quoted for patients in the Western world and those elsewhere in Africa. In two African series from Zimbabwe and Kenya, the mean age at presentation was 54.8 years and 52.5 years respectively (Mukiibi and Mkwananzi, 1987; Mukiibi et al, 1981). Consistent with this is the higher percentage of patients in African series who present under the age of 40 years - 19.4% and 8.3% of the patients seen by Mukiibi et al (1981) and Mukiibi and Mkwananzi (1987) respectively, were <40 years old, while 7.1% of our patients were <40 years of age (see Table 3.1). This is in contrast to the much lower figure of 2% reported by Kyle (1975). A slight male excess is seen in the vast majority of series, irrespective of ethnic group and location.

The major symptoms at presentation are noted in Table 3.2. Bone pain was the commonest symptom, occurring in 94.1% of patients. In other series, bone pain has been documented as being present in 87% (Talerman, 1969), 85% (Mukiibi and Mkwananzi, 1987), 68% (Kyle, 1975) and 63% (Kapadia, 1980). Other noteworthy symptoms include those related to anaemia (weakness, fatigue, etc. - 81.2%) and infection (59.8%). Bleeding was uncommon (9.5%), compared to anaemia and infection. Fever and documented infection were commoner in our patients, the prevalence being higher than the 12% noted in the series of Kyle (1975).

Anaemia was the dominant clinical sign (82.4%). Plasmacytomas were evident in 40.6%, while pathological fractures were seen in 14.8% of the patients (see Table 3.3 and Figure

3.1). Plasmacytomas are usually seen in the context of systemic myeloma. Less than 10% of our patients with plasma cell disorders had a solitary plasmacytoma. This is in contrast to the Malawian series reported by Tozer et al (1980), where the presentation was most often with a localised tumour mass in bone (20/29 patients), and the report of Jacobson et al (1975), who estimated that approximately 15% of black South African patients with myeloma present initially with solitary myeloma of bone. Classical lytic bone lesions remain the radiological hallmark of myeloma (see Figures 3.2 and 3.4). They were present in 88.4% of our patients, 70% of patients in the series of Kyle (1975) and 65% in the series of Mukiibi and Mkwananzi (1987). Other radiological features included osteoporosis, vertebral compression fractures (50.3%) and pathological fractures (14.8%) (see Figures 3.3 and 3.4). The high proportion of vertebral compression fractures seen in our patients was also noted by Shulman and Jacobson (1980). They concluded that the increased prevalence of vertebral fractures in black South African myeloma patients may be explained by the associated increased frequency of osteoporosis and haemosiderosis in the black population.

Table 3.4 shows the variable performance status (PS) at presentation, with 56.5% having a PS of 1 or 2 and 43.5% a PS of 3 or 4. A feature common to patients seen at our hospital, is late stage presentation, with advanced stage disease. Based on the Durie and Salmon staging system (1975), the majority of patients with myeloma presented with stage III disease (74.6% - see Table 3.5).

A serum paraprotein on electrophoresis was noted in 83.5% of the patients, while a urine paraprotein was seen in 85.9% of patients. Kappa light chains (63%) were roughly twice

as common as lambda light chains (37%). The distribution of the serum and urine paraprotein is similar to other series (Acute Leukemia Group B, 1975; Kyle, 1975). IgG myeloma was the commonest isotype (62.9%), followed by IgA myeloma (19.4%) and light chain disease (12.4%). IgD myeloma is rare (1.2%). There were no patients with IgE or IgM myeloma. 1 patient (0.6%) had nonsecretory myeloma (see Table 3.6). In 60 of the 76 patients of Mukiibi and Mkwananzi (1987), who had an immunoglobulin profile performed, 81.7% had IgG myeloma and 18.3% IgA myeloma respectively. A surprising finding is the high proportion of IgM myeloma (25%), reported in a Nigerian study by Onyemelukwe and Kulkarni (1988). The remainder of their patients had IgG (64%) and IgA (11%) myeloma.

The laboratory characteristics are shown in Table 3.7. Anaemia is a classical feature of myeloma. The mean haemoglobin (Hb) at presentation was 9.38g/dl. Anaemia was present in 89.9% of females (Hb <12g/dl) and 77.2% of males (Hb <13g/dl). A Hb of <8.5g/dl was seen in 42.4%, while a Hb <10g/dl was noted in 62.4% of the patients. Kyle (1975), found that 62% of patients showed a Hb <12g/dl and 8% had a Hb <8g/dl. Anaemia (Hb <11.5g/dl) was present in 68% of patients in the series of Mukiibi and Mkwananzi (1987) , while Talerman (1969) noted a Hb of <9g/dl in 50% of his patients. In contrast to the low Hb, the white cell count and platelet count was usually normal. Only 9.5% and 3.6% of patients manifested with a leucopenia (wcc <4 x 10^9/l and <3.4 x 10^9/l respectively), while 16% presented with a leucocytosis (wcc >11 x 10^9/l). Thrombocytopenia (platelets <100 x 10^9/l) and thrombocytosis (platelets >400 x 10^9/l) were equally present in 10.1% of individuals. Leucopenia (wcc <4 x 10^9/l) was present in

16%, while thrombocytopenia was noted in 13% of patients in the series of Kyle (1975). Leucopenia and thrombocytopenia were associated with advanced disease and a worse prognosis.

The ESR, although regarded as a non-specific investigation, is raised above 20mm/hr in the vast majority of patients (92.6%). The mean ESR was 106 mm/hr. A markedly elevated ESR of >100mm/hr was present in 64.4% of the patients. A normal ESR in the correct context does not exclude myeloma, but should indicate the possibility of light chain myeloma. An elevated total protein of >85g/l was found in 66.9% of myeloma patients. The mean total protein in our patients was 98.44g/l. A similarly high mean total protein of 91g/l and 96g/l was noted by Mukiibi and Mkwananzi (1987) and Mukiibi et al (1981) respectively. A total protein in excess of 100g/l was present in 39.8% of the patients and is a useful clue to the diagnosis of myeloma in middle aged and elderly individuals. The mean IgG, IgA and IgM levels were 45.63g/l, 9.57g/l and 0.79g/l respectively. Although these results refer to our myeloma patients, it is interesting to note that higher serum immunoglobulin levels have been noted in black South African adults compared to their white counterparts (Shulman et al, 1975). In the present series of 170 patients, 74.5% had a monoclonal protein of >10g/l, while in 51.5% of patients, the paraprotein was >30g/l. Typically, myeloma patients have an elevated globulin level, but may also show a decreased albumin level. The mean globulin level was 65.31g/l, in contrast to 36g/l (Kyle, 1975). A level of >30g/l was noted in 89.8% of individuals. In our patients, the albumin level was <35g/l in 60.2% and <30g/l in 33.1% respectively. In some series, a low albumin has been shown to be an adverse prognostic factor (Bladé et

al, 1989; Mukiibi and Mkwananzi, 1987). Moreover, if the calcium level is uncorrected in the face of a low albumin, hypercalcaemia may be underestimated.

Hypercalcaemia is an important feature of myeloma. The mean corrected calcium level in the present series was 2.78mmol/l. 39.8% of patients have a corrected calcium level above 2.65mmol/l, while a level above 2.85mmol/l was found in 31.3%. A calcium of >3mmol/l was present in 22.9% of patients on admission. In a series from St. Bartholomew's Hospital, corrected calcium levels above 2.67mmol/l were seen in 26% of patients (Malpas et al, 1995). Hyperuricaemia was a common finding, being present in almost 2/3 (60.6%) of our patients. Renal impairment (persistently elevated urea of >8mmol/l and a creatinine of >180μmol/l, after rehydration) was evident in 27.7% and 15.7% of patients respectively. The majority of patients with myeloma had a normal alkaline phosphatase level (80.1%), in keeping with the non-osteoblastic nature (osteoclastic) of the bone disease in myeloma. LDH levels were raised in 45.2% of patients at presentation. Beta 2 microglobulin levels above 6mg/l were noted in 40.1% of individuals, while the CRP, a surrogate for IL-6 was raised above 12mg/l in 50.8% of patients. A T_4 lymphopenia was present in 30.8% of the patients, while a T_8 lymphocytosis was evident in 22.2 %. A subnormal T_4:T_8 ratio of <1 was found in 42.5% of the patients. Only 9.6% of the patients had a subnormal vitamin B_{12} level. A bone marrow plasmacytosis >10% was noted in 69.1% of individuals. 32.4% had levels between 10% and 30%, while a marrow plasmacytosis of >30% (a major diagnostic criteria for myeloma) was seen in 36.7% of patients (see Figure 3.5).

Table 3.8 shows the parameters of prognostic significance in the 170 patients. The mean survival in months is noted with each of these parameters and the corresponding statistically significant p value is also included in Table 3.8. Poor prognostic factors may be related to the intrinsic malignancy e.g. CRP, or reflect tumour burden e.g. beta 2 microglobulin. Poor prognostic factors of statistical significance in our patients based on a univariate analysis included: a beta 2 microglobulin of >6mg/l (both uncorrected and corrected for renal impairment), hypercalcaemia >2.65mmol/l, urea >8mmol/l, creatinine >180μmol/l, CRP >12mg/l, IgG >50g/l, platelets <100 x 10^9/l, serum paraprotein >10g/l, intermediate and advanced stage disease (especially stage III B), urine total protein >1g/l and a white cell count of <4 x10^9/l (especially <3.4 x 10^9/l). An ESR <20mm/hr is a favourable prognostic sign. The serum calcium, paraprotein, immunoglobulin levels, urinary protein and impaired renal function are well recognised adverse prognostic factors in myeloma. Most of these form the basis of the Durie and Salmon staging system (Durie and Salmon, 1975). Interestingly, anaemia was not an adverse prognostic factor in our patients. Also, the percentage of plasma cells in the bone marrow, LDH level and immune dysregulation did not confer an adverse prognosis. Leucopenia and thrombocytopenia when present in our patients, are associated with an adverse prognosis.

Beta 2 microglobulin levels are highly predictive of survival (Durie et al, 1990; Simonsson et al, 1988; Bataille and Grenier, 1987). Serum levels correlate with tumour stage, but may not do so, if the levels are corrected for the degree of azotaemia (Brenning et al, 1986). In our patients, both the uncorrected and corrected values were

statistically significant. CRP levels correlate with disease activity and prognosis (Omede and Pileri, 1991; Bataille et al, 1989). When coupled with the beta 2 microglobulin levels, it appears to be highly predictive with respect to prognosis (Greipp et al, 1993; Bataille et al, 1992).

All the patients were treated with conventional chemotherapy. The standard chemotherapy regimen used initially in the vast majority of patients was melphalan and prednisone. For refractory disease, combination chemotherapy with vincristine, an anthracycline and dexamethasone was used in most of the patients. None of the patients were subjected to peripheral stem cell or bone marrow transplantation. The overall mean survival for the 170 patients was 28.37 months, with a lower median survival of 15.5 months (see Figure 3.6). The lower median survival reflects the higher proportion of early deaths in our patients, consequent on the late presentations with advanced stage disease and often a multiplicity of problems (complications).

4.2 CASE-CONTROL STUDY

130 cases (patients) and 130 matched controls were interviewed prospectively, to determine risk factors for myeloma. In both the groups, 76 (58.5%) were males and 54 (41.5%) were females, with a male to female ratio of 1.45:1. The mean age at presentation was 61.5 years (range 30-91 years) for the cases and 59 years for the controls (range 35-87 years). 6.1% of the patients were <40 years of age (see Table 3.9). The clinical, radiological and laboratory characteristics as noted in Tables 3.10 to 3.15 are very similar to the corresponding data on the 170 patients discussed in section 4.1.

Prognostic factors of statistical significance based on a univariate analysis in the 130 patients included: a beta 2 microglobulin of >6mg/l (both uncorrected and corrected for renal impairment), urea (>8mmol/l), creatinine (>180μmol/l), IgG >50g/l, platelets <100 x 10^9/l, serum paraprotein >10g/l, intermediate and advanced stage disease (especially stage III B), and urine total protein >1g/l. Compared to the larger group of 170 patients, hypercalcaemia, leucopenia and an elevated CRP were not statistically significant adverse prognostic factors (see Table 3.16).

The overall mean survival for the 130 patients was 31.95 months, with a lower median survival of 20 months (see Figure 3.7).

Further discussion with regard to the case control study will focus on the risk factors that were looked at in the interview questionnaire (see appendix A), in both the cases and controls.

4.2.1 Radiation exposures

Radiation exposures are considered to be one of the more convincing risk factors for myeloma. Radiation exposures include exposure to the atomic bomb, radiation related occupations such as nuclear workers and radiologists, and recipients of therapeutic and diagnostic radiation. Ionizing radiation is potentially carcinogenic. The target of ionizing radiation is believed to be DNA, or more precisely the proto-oncogenes in DNA.

Questions with regard to type of radiation exposure (particularly in relation to work) , duration of radiation exposure and receipt of therapeutic and diagnostic radiation were asked. None of the patients and 3 controls were exposed to radiation in the work

environment. 109 controls and 77 patients received diagnostic radiation (mostly in the form of plain X-rays). Diagnostic radiation in this study implied radiation received (for diagnostic purposes) prior to the present hospital admission. None of the patients had prior radiotherapy (therapeutic radiation), while 2 of the controls received radiotherapy (one for carcinoma of the lung and the other for carcinoma of the cervix) (see Table 3.17). Thus, radiation exposure was not a risk factor for myeloma in our patients.

4.2.2 Occupational and environmental exposures

A number of occupational and environmental exposures are associated with an increased risk of myeloma. Most of these are in relation to agriculture (farming), but the list includes exposure to metals, asbestos, benzene and petroleum, paints and solvents, rubber, wood, leather, food processing and a host of miscellaneous occupational exposures (see chapter 1).

The exact role of occupational exposures as a risk factor in myeloma remains uncertain.

In the questionnaire (see appendix A), questions were asked regarding the occupational history (e.g. job title, description of work done, duration of employment, etc.), specific questions with respect to farming and agriculture (such as type of farm, description of work done, exposure to pesticides and herbicides, exposure to live animals, duration of exposure, etc.) and exposure to specific occupations.

There are a number of shortcomings with respect to eliciting the past history in both patients and controls. All the previous exposures may be difficult to recall. Also, it is possible that agents known to be potentially hazardous may be better recalled by cases

than controls. Another problem is that the specific chemical or physical agent may be known to the respondents by different names. Additionally, in many of the occupations, exposure to a wide variety of agents occurs, making causal associations to specific chemical and physical agents less clear. There is also the added problem of interviewer bias, especially where there is a clear knowledge (by the interviewer) of the known risk factors associated with the disease.

A mention will be made of the dominant occupations in both the cases and controls. This will be followed by a review of the findings with respect to farming and agriculture and exposures to specific agents (see Table 3.17).

There were 86 different types of dominant occupations (i.e. the type of work that the individual spent most of his time doing) in both the cases and controls. The 12 dominant occupations in descending order are shown in Table 3.29.

4.2.2.1 Agriculture

It has been pointed out earlier in section 4.2, that a significantly increased risk for myeloma in agriculture (particularly farming), has been reported in a number of studies. Fewer studies have shown no association.

In the reports in which an association has been suggested, because of the wide variety of different exposures, it is often not possible to determine whether oncogenic zoonotic viruses, pesticides (including insecticides and herbicides), agricultural chemicals or some combination of these exposures is responsible for the increased risk of myeloma.

The findings in our study concur with the reports in the literature. A statistically significantly higher number of cases (93 - 71.5%) compared to controls (67 - 51.5%), spent time at a farm (p=0.0009; odds ratio 2.363). More of the cases spent time as the owners or workers on a farm, compared to the controls (p=0.008). The number of cases (66) who worked on a farm was significantly higher than the control group (28) (p=0.00005; odds ratio 3.683). However, it was not possible to determine the exact nature of the increased risk or the factors that contributed to the increased risk. There was no significant difference between the cases and controls with regard to animal exposure (cases 58, controls 27; p=0.635), pesticide exposure (cases 20, controls 9; p=0.995) or exposure to exhaust fumes (cases 13, controls 6; p=0.945), although in each of these categories the exposures were more in the cases than controls. Within the cases, there was no significant difference with respect to gender (p=0.152). Furthermore, the geographical location of the farm (including the type of farm) did not differ significantly between the cases and controls (p=0.537).

4.2.2.2 Metals

Exposure to various metals have been linked with the development of myeloma. 7 cases and 6 controls in the present series were exposed to lead. Although the number is slightly higher in the cases, the difference was not significant (p=0.765; odds ratio 1.186).

No exposures with regard to other metals such as arsenic, cadmium, chromium and nickel were noted in either the cases or controls.

4.2.2.3 Rubber manufacturing

Rubber manufacturing has been incriminated as a risk factor for myeloma (Cuzick and De Stavola, 1988). Rubber manufacturing may allow exposure to a variety of substances, including: organic solvents, plastic monomers, rubber additives and asbestos. In the past, exposure to benzene was high. 8 controls and 5 cases were involved in rubber manufacturing (p=0.393).

4.2.2.4 Benzene and petroleum

Benzene and its metabolites are known to be marrow toxic (Decouflé et al, 1983). Benzene has been used as a solvent by painters and in various industries in which myeloma excesses have been noted. Benzene exposure has been associated with myeloma (Goldstein, 1990). However, more recent studies do not indicate a positive association between myeloma and benzene (Bergsagel et al, 1999; Savitz and Andrews, 1997; Bezabeth et al, 1996). In the present study, a higher non-statistically significant number of cases (11) were exposed to benzene, compared to controls (6) (p=0.209; odds ratio 1.910).

4.2.2.5 Miscellaneous occupational exposures

Positive associations between myeloma and a wide variety of other occupational exposures have been reported. Many of these occupational exposures were explored in the case-control study interview. The findings are noted in Table 3.17.

Exposures with respect to asbestos, fibreglass/mineral fibres, mining, or working at a building construction site, as a caterer/cook or in the forestry industry were all higher in the cases compared to controls. However, none of the exposures were statistically significantly different.

The following exposures were found to be higher in the present study in the controls compared to the cases, viz., chemicals and dyes, batteries, electricity, welding, work as a butcher, baker/confectioner, food processor, painter, plasterer, work in the furniture and carpentry industry and in the medical and pharmaceutical industry.

Exposures to coal tar, heat and electrical insulation, work at a power station, work as a smelter and printer were found to be equally present among the cases and the controls.

4.2.3 Non-occupational exposures

4.2.3.1 Medicines

Medications have been suggested as myeloma risk factors in several case-control studies. Some of the specific drug exposures that were looked for in the study will be mentioned (see Table 3.17). Additionally, drugs that were commonly used on a chronic basis by the cases and controls were also noted. The chronic use of medicines was more common in the controls than in the cases. Although a number of different drugs were used, diuretics, anti-hypertensives, oral hypoglycaemics, analgesics including non-steroidal anti-inflammatory drugs and vitamins were the most frequently encountered.

The specific drug exposures included appetite suppressants, benzodiazepines, beta-blockers, cytotoxics, diphenylhydantoin, herbal toxins, ibuprofen, laxatives,

nitroglycerine and phenobarbitone. Use of herbal toxins, ibuprofen and laxatives was found to be higher in the cases than in the controls, but these higher numbers were non-significant.

4.2.3.2 Smoking

The findings in this study with respect to smoking are similar to most other studies which have shown no relationship between tobacco use and myeloma occurrence. There were fewer cases (59 - 45.4%) who smoked, compared to controls (72 - 55.4%) (p=0.106; odds ratio 0.669).

4.2.3.3 Alcohol

Alcohol consumption has generally not been shown to be associated with myeloma. Similarly, no association was found in the present study. 61 cases (46.9%) consumed alcohol, while a higher figure was noted among the controls (76 - 58.5%).

4.2.4 Pre-existing medical conditions/chronic antigenic stimulation

Chronic antigenic stimulation (CAS) of the immune system has been suggested as a risk factor in the development of myeloma. This is based on the simple assumption that antigenic stimulation in a susceptible host is the first hit, of the so called 'two-hit hypothesis', giving rise to a benign monoclone. The second hit is postulated to be a mutagenic or transforming event that gives rise to myeloma from the expanded monoclonal B-cell population.

CAS may also cause progression of disease. This occurs via the cytokine interleukin-6 (IL-6) (see chapter 1). IL-6 is produced by many cell types in response to a number of stimuli including bacterial products, viruses and trauma (Wolverkamp and Marquet, 1990). IL-6 production has also been noted in other diseases, such as rheumatoid arthritis, systemic lupus erythematosus, trauma, acute infectious neural disease, cardiac myxoma and transplantation (Wolverkamp and Marquet, 1990).

Another pathway by which CAS could be associated with myeloma is through production of a monoclonal protein. The elaboration of a monoclonal protein has been noted with infections, chronic inflammatory disorders, malignancy, neurological and dermatological diseases, chronic liver disease, post renal transplant immunosuppression and a number of miscellaneous conditions (Passweg et al, 1996; Bladé and Kyle, 1995; Merlini and Aguzzi, 1998; Saleün et al, 1982).

Epidemiological studies have explored the role of CAS by utilising medical history information, in particular, past exposures to viral and bacterial illnesses, childhood immunization, allergies and autoimmune disease. Similar information has been explored in the case-control study and will be detailed below (see also Table 3.17).

Allergies were more commonly encountered in cases (8), compared to controls (4) (p=0.4129; odds ratio 2.083). Chronic childhood illnesses were evident in 16 controls and 12 cases. Viral infections occurred with a variable frequency in both groups. Measles was slightly commoner in the cases (17) than controls (15) (p=0.047), as was mumps cases-(7) and controls-(6); p=0.0196. 18 controls and 11 cases had chicken pox (p=0.0189). It should be noted that despite the small differences in numbers between the

cases and controls in these three viral infections, the p values are statistically significant. These significant values result from the almost equivalent number in the 'unknown category' in the cases, and should therefore be interpreted with caution.

Tuberculosis and asthma were significantly higher in the controls (p=0.0035 and p=0.0053), both of these being common disorders in the general population. Malaria, chronic bronchitis and whooping cough were non-significantly elevated in the controls compared to the cases. None of the cases or controls had systemic lupus erythematosus, rheumatoid arthritis, chronic otitis media or recurrent urinary tract infections. Prior surgical operations were performed in 50 cases and 45 controls (p=0.550).

It is interesting to note that a higher number of controls (123) were immunized against the common childhood illnesses, compared to cases (104). The difference is statistically significant (p=0.0019). Thus, immunization may play a protective role against myeloma.

The role of viruses, in particular the role of human herpesvirus-8 will be discussed later. A brief mention will be made of the association between HIV and myeloma. In general, the incidence of myeloma does not appear to be increased in patients with HIV/AIDS. However, a recent study by Goedert et al (1998), reports a 4.5 fold increase in myeloma among people with AIDS. The increase was noted mainly in the post-AIDS period (3 months to 27 months after the diagnosis of AIDS), rather than in the pre-AIDS phase of the disease. Also, 10 of the 16 patients with myeloma were homosexual males. The HHV-8 seroprevalence in this group is not known. It is possible that coexisting infection with HHV-8 may explain this interesting observation.

There are other reports of the two diseases occurring in the same individual, whether it be coincidental or causal (Nogues et al, 1996; Nosari et al, 1996; Piras et al, 1996; Kumar et al, 1994). Myeloma in association with HIV infection occurs in younger patients. Moreover, the clinical presentation appears to be atypical and unusual (Piras et al, 1996; Schulz et al, 1996; Kumar et al, 1994). Plasmacytomas are common, as is localisation in other extramedullary sites. Non-secretory tumours are commoner than in the seronegative patient. Overall, the prognosis and clinical outcome is also less favourable.

Two of the 130 cases (or even 170 patients with myeloma, as there were no other seropositive patients in the additional 40 patients) were seropositive. The first patient was a 51 year old male who presented with bone pain and a plasmacytoma affecting the left mandible, maxilla, buccal tissues and nasopharynx. He had no anaemia or hypercalcaemia. His renal function was normal. Similar to the description in the literature, he had a non-secretory myeloma. The skeletal survey demonstrated extensive lytic bone disease. His bone marrow showed 10% plasma cells. His CD4 count was 90 x 10^6/l. He had no evidence of an opportunistic infection. He was treated with local radiotherapy and dexamethasone. The plasmacytoma regressed. He was lost to follow up 3 months after admission.

The second patient was a 38 year old female who presented with severe anaemia (Hb=2.2g/dl). She had no hypercalcaemia or renal impairment . She had an IgG λ isotype (paraprotein of 51,5g/l) and a bone marrow plasmacytosis of 14%. Her CD4 count was 180 x 10^6/l. She was transfused with packed cells, commenced on erythropoietin and

received oral chemotherapy. Despite these measures, the anaemia proved to be refractory. She was lost to follow up 6 months after diagnosis.

4.2.5 Socioeconomic and other influences

The influence of socioeconomic status as a risk factor for myeloma is unclear. A number of studies looking at various parameters of socioeconomic status (including income, educational level, occupation and social class, home ownership etc.) are reported in the literature (see chapter 1). Both positive and negative associations have been documented.

A number of parameters were looked at in the case-control study. This included different aspects such as the present address (where person lives), birth place, where individual lived most of his life, tribe/language, marital status, home ownership, highest monthly nett income, type of home, number of persons per room, education and occupational rank (social class) (see Tables 3.18 to 3.28).

The present address was found to be statistically significantly different between the cases and controls (p=0.0016). Most of the controls (122 - 93.8%) had a Gauteng address, with a smaller number presently living elsewhere. 94 cases (72.3%) gave a Gauteng address, while the remaining 27.7% came from outside of Gauteng. This is to be expected, as a number of cases were referred from outside of Gauteng, given the specialised nature of the treatment required for myeloma. With respect to the birth place, a smaller number of the controls (65 - 50%), were born in Gauteng. Although this is higher than in the cases (44 - 33.8%), it is not significantly different when compared to

the controls (p=0.1923) (see Tables 3.18 and 3.19). Interestingly, in the third category of lived most, there is a significant difference between the cases and the controls (p=0.00258) (see Table 3.20). Significantly more controls 'lived most' in the Gauteng area compared to controls.

No significant difference was found in the tribe/language between the cases and controls (p=0.5270) (see Table 3.21). Zulu, Sotho, Tswana and Xhosa were the most frequently encountered tribes/languages in both groups.

Marital status was also looked at. It was surprising to note that there were significant differences between the cases and controls (p=0.0057). Most of the cases were married (94 -72.3%), compared to a lesser number of the controls (65 - 50%).

No significant differences were noted in relation to highest income (p=0.4272), home ownership (p=0.8954), number of persons per room (p=0.6313) or type of home (p=0.2760) (see Tables 3.23 to 3.26).

Also, the educational status (p=0.1710) and occupational rank (social class) (p=0.4543) were similar in the cases and controls (see Tables 3.27 and 3.28).

Therefore, the above findings suggest that the indices of socioeconomic status does not appear to have an influence on myeloma risk in our patients.

Kaplan-Meir survival analyses were performed on the various parameters that were studied in the interview questionnaire, with respect to the 130 cases. Statistically significant survival differences were noted with regard to the following parameters, viz.,

i) Asbestos (p=0.0120). Strangely, those cases exposed to asbestos had a longer mean survival of 40.94 months, compared to those who were not exposed (mean survival

31.28 months), ii) work at a building construction site (p=0.0336). The mean survival was much lower in those exposed (12.54 months), compared to those who were not exposed (34.24 months), iii) immunization (p= 0.0013). It is noteworthy that individuals who were immunized survived longer (mean survival 36.92 months) than those who were not immunized (mean survival 28.33 months), iv) lived most (p=0.00005). It is interesting to note that individuals who lived most of their lives further away from the treatment centres at Gauteng, had a shorter survival. This shorter survival may be related to less access or delayed access to sensitive diagnostic methods and adequate medical treatment. The greater distance may also imply less compliance on the part of the patient with regard to regular follow up assessments. The inability of some patients with a poorer performance status to travel long distances may be an added factor, similar to that pointed out by Lenhard et al (1987), in their study of USA myeloma patients. Immunization was probably more readily available in the urban areas and may account for the higher numbers immunized in the control group, and v) type of home (p=0.0168). The mean survival (33.68 months) was much higher in those individuals who lived in a brick house, compared to those who lived in another type of dwelling such as a shack (mean survival 16.63 months). However, this is difficult to explain as the survival was not significantly different with respect to other parameters of socioeconomic status such as number of persons per room, income, home ownership, education and occupational rank. Another explanation could be that the numbers in the groups other than category one (brick house) are small, making the statistical significance less meaningful.

4.3 HLA STUDY

Evidence that genetic factors play a role in the pathogenesis of plasma cell tumours, is based on striking differences in the incidence f monoclonal gammopathy and plasmacytomas in different inbred strains of mice (Potter et al, 1975; Radl and Hollander, 1974), racial differences in the incidence of MGUS and myeloma in humans (Riedel and Pottern, 1992), the association of an increased risk of developing myeloma with certain human leucocyte antigens (HLA) (Pottern et al, 1992b) and the occurrence of familial myeloma (see chapter 1).

The class I and class II antigens in 62 myeloma patients were analyzed and compared to 100 ethnically matched controls (except in the case of antigen frequencies involving the A locus, where there were 99 controls).

The clinical, radiological and laboratory characteristics are shown in Tables 3.30 and 3.31. The findings are similar to the 170 and 130 patients discussed earlier, and will therefore, not be elaborated upon.

The antigen frequencies and the haplotype frequencies in the patients and controls are shown in Tables 3.32 to 3.38. HLA associations that have been published in myeloma patients include: A3, A5, B5, B18, Bw65, Cw2, Cw5, Cw6 and DRw14 (see Table 3.39). Significantly higher gene frequencies for Bw65, Cw2 and DRw14 are reported in American blacks with myeloma, while white cases had higher gene frequencies for A3 and Cw2. The frequency of Cw2 in the black and white controls was similar. These findings suggest that the Cw2 allele, or a gene close to the C loci, confers susceptibility to the development of myeloma, but does not explain the higher risk among blacks. In a

recent study, B13 was found to be statistically significantly increased in myeloma patients with active disease (Zaraisky and Podoltseva, 1998). In another study among American blacks, an association was found with the HLA-Cw5 antigen (4 of 22 cases, compared to 2 of 138 controls; OR=15) (Leech et al, 1983). All four myeloma cases with the Cw5 antigen were males. In the same study, a relationship was also found with the Cw6 antigen (OR=6.5).

In the present study, the corresponding association found was B18. Cw5 occurs at very low frequency in South African blacks (Wadee and Dunn, 1991). Additional HLA types in which the frequencies are higher in patients compared to controls include: A1 (relative risk-RR=1.3), A2 (RR=1.3), A23 (RR=1.4), A24 (1.1), A30 (RR=1.2), A34 (RR=4.9), A43 (RR=8.2), B22 (RR=4.9), B35 (RR=11.8), B41 (RR=4.9), B42 (RR=1.1), B45 (RR=1.7), B70 (RR=1.1), DR3 (RR=1.3), DR8 (RR=3.8), DR10 (RR=2.1), DR11 (RR=1.3), DR12 (RR=3.8) and DQ3 (RR=1.4). Of the additional HLA types, only HLA B35 was found to be statistically significantly different (p value=$0.02<p<0.05$). The relative increased risk for myeloma in individuals with HLA B35was 11.82. Some of these additional HLA types were only detected in myeloma patients. This includes the following: A34, A43, B22, B35 and B41. B22 is absent in South African blacks, while A34 and A43 occur in low frequency in South African caucasians (Wadee and Dunn, 1991).

Antigens found only in the controls) include: A25, B38, B39, B51, B63 and DR9. A25 is virtually absent, or detected infrequently in the South African caucasians , and has a low frequency in South African blacks (Wadee and Dunn, 1991; Du Toit et al, 1988). B38 is

also not detected or detected infrequently in South African blacks (Wadee and Dunn, 1991; Du Toit et al, 1988).

Haplotype frequencies were also studied. The haplotype frequencies which conferred a relative higher risk and those associated with a lower risk (i.e. they may be protective) are shown in Tables 3.37 and 3.38 respectively. Of these haplotypes, those that are commonly found in South African blacks include A30,Bw42; Bw42,DR3 and B70,Dw3 (Du Toit et al, 1988).

The HLA class I and classII antigen and haplotype frequencies were not correlated with the clinical parameters, as the numbers in individual groups were generally small. The findings, however, are interesting and require further study.

4.4 HHV-8 STUDY

The results of the patient characteristics, bone marrow and serological findings are depicted in Table 3.40. Kaposi's sarcoma herpesvirus/human herpesvirus-8 (KSHV/HHV-8) is a recently implicated environmental factor that may play a role in the pathogenesis of myeloma (see chapter 1). Using a nested polymerase chain reaction (PCR) assay, we tested for the presence of KSHV DNA sequences in bone marrow aspirates, bone marrow biopsy material and cultured bone marrow adherent cell samples of myeloma patients.

KSHV DNA sequences were detected in 4/10 (40%) of the adherent cell cultures and 1/20 (5%) of the bone marrow aspirate samples (see Figures 3.8 and 3.9). None of the bone marrow biopsy samples (0/9), or control bone marrow aspirate samples were positive.

In the study of Rettig et al (1997b), KSHV DNA sequences were detected by PCR in the dendritic cells of 15/15 patients with myeloma and 2/8 patients with MGUS. No KSHV DNA sequences were present in the malignant plasma cells or bone marrow dendritic cells from normal individuals (0/10) or patients with other malignancies (0/16) (Rettig et al, 1997b). KSHV encodes an IL-6 homologue (Moore et al, 1996), and IL-6 is known to be an important growth factor for myeloma cells (Klein et al, 1995). IL-6 is produced by adherent cells (dendritic cells) in the microenvironment of the bone marrow. Dendritic cells have been shown to play a central role in the growth and differentiation of mature B-cells and to increase (by 30- to 300- fold), the secretion of immunoglobulins G and A by B-cells (Dubois et al, 1997). KSHV infection of dendritic cells localises the virus to

the bone marrow microenvironment where viral genes (such as the gene encoding vIL-6) are expressed and may support myeloma growth. vIL-6 RNA transcripts were present in 3/3 KSHV infected bone marrow stromal cell samples from myeloma patients (Rettig et al, 1997b), and may therefore play an important role in producing paracrine stimulation of plasma cell growth and maintain abnormal plasma cell proliferation in myeloma and related plasma cell disorders.

The findings of Rettig et al (1997b), are supported by two further studies in which KSHV was detected in 12/25 (48%) and 2/3 (67%) respectively of cultured bone marrow dendritic cells (Agbalika et al, 1998; Tisdale et al, 1997). Our study provides further evidence of the presence of the virus in 4/10 (40%) of cultured bone marrow adherent cells. Similar to the latter two studies, our detection rate is much lower than that described originally. Possible explanations are that there may be differences in the sensitivity of the primers used in different studies, or that the virus may not be universally present or involved in the aetiopathogenesis of the disease. Furthermore, KSHV could be under a strict T-cell mediated control in myeloma patients, leading to a very difficult detection by sensitive PCR (Tarte et al, 1998). Also, with respect to our findings, could the detection rate reflect the possible high background seroprevalence rate?

Mitterer et al (1998), in a recent study were unable to dete . the KSHV genome in a serum-free media of cultured dendritic cells, as opposed to the demonstration of the virus from dendritic cells in prior studies. Olsen et al (1998), provide further evidence against the involvement of the virus in myeloma. In their study, all the bone marrow biopsies (0/16) and negative controls (0/14) were vIL-6 negative.

The demonstration of the virus in leukapheresis products has been less successful. In four studies, KSHV was not detectable or was present in only a small proportion of patients (Cull et al, 1998; Tarte et al, 1998; DeGreef et al, 1997; Masood et al, 1997). One study however, did confirm the presence of the virus in leukapheresis products (Vescio et al, 1997).

The detection of KSHV in core bone marrow biopsies by PCR and ISH was shown in 17/20 (85%) of myeloma patients by Said et al (1997). Their results were corroborated by two studies in which the virus was detected in 5/10 (50%) and 18/20 (90%) of patients respectively (Agbalika et al, 1998; Brousset et al, 1997). Two further studies, using either Southern blotting of unnested PCR amplification products or nested PCR, failed to amplify the KS_{330} sequence (ORF 26) in bone marrow biopsies from myeloma patients (Cathomas et al, 1998; Olsen et al, 1998). In another study, Dupin et al (1999), did not observe any staining for HHV-8 LNA-1 (Latent nuclear antigen-1) from bone marrow biopsies in myeloma. In our study, we were unable to detect the presence of the KSHV DNA sequences in core bone marrow biopsy samples (0/9).

With regard to bone marrow aspirates, most of the studies proved to be negative (Dubois et al, 1997; Parravicini et al, 1997; Rettig et al, 1997b). We detected a positive result in 1/20 (5%) of the patients in our study. The significance of this low positive yield is unclear. In general, the poor yield in fresh bone marrow aspirates is likely to be due to the low number of stromal cells, heavy contamination by peripheral blood, presence of up to 90% uninfected neoplastic plasma cells and insensitivity of the PCR assay (Rettig et al, 1997b).

KSHV is not ubiquitous in humans (Kedes et al, 1996). Antibodies to other herpesviruses which are ubiquitous have been detected in a high proportion of patients with myeloma (Whitby et al, 1998; MacKenzie et al, 1997; Marcelin et al, 1997; Masood et al, 1997; Parravicini et al, 1997), thus arguing against the notion that myeloma patients are unable to mount an adequate immunological (antibody) response. The overall seroprevalence rate is less than 5% in the US and UK, up to 35% in southern Italy, and over 50% in East Africa (Whitby et al, 1998; Gao et al, 1996; Kedes et al, 1996; Miller et al, 1996; Simpson et al, 1996). The seroprevalence rate in the general population in South Africa is likely to be intermediate between the low rates in the US and UK and the very high rates in East Africa. In a recent study of rural South African patients attending the Hlabisa Hospital, KwaZulu-Natal, the overall seroprevalence rate of 136 patients (including 50 patients with a sexually transmitted disease, 50 adult medical ward patients and 36 paediatric ward patients) was 34.6% (Wilkinson et al, 1999). In this study, antibodies to the latency-associated nuclear antigen (LANA), were measured by immunofluorescence. In another local study, the prevalence of anti-HHV-8 antibodies was studied in a large population of black South African cancer patients, including Kaposi's sarcoma. The seroprevalence of antibodies to HHV-8 was 32% in 3293 subjects with cancer (excluding Kaposi's sarcoma), while the seroprevalence rate was 83% in 51 subjects with Kaposi's sarcoma. For the 108 patients with myeloma (included in this study), the seroprevalence rate was 24% (Sitas et al, 1999). Antibodies to HHV-8 were detected using an indirect immunofluorescence assay for the latent ORF73 antigen (LNA-1). Interestingly, the patients in this study were from the three

main teaching hospitals in Johannesburg and Soweto, including Chris Hani Baragwanath Hospital (where our study was conducted).

Presently, it appears that the association between myeloma and KSHV is not universal and remains unclear. Nevertheless, attempts at defining the exact link between KSHV and myeloma are of particular relevance and importance. Implications include the use of appropriate therapeutic strategies such as antiviral agents and vaccines in the management of patients with myeloma. Feasibility studies evaluating vaccination strategies (using idiotype protein pulsed dendritic cells) are being done (Reichardt et al, 1996). This may need to be reviewed in the light of the finding of KSHV in dendritic cells of myeloma patients.

Further studies may clarify the precise role of KSHV in myeloma, and provide a clearer understanding of myeloma oncogenesis.

4.5 FLOW CYTOMETRY STUDY

The results of DNA ploidy in myeloma patients in the literature shows marked variability with regard to both the frequency of aneuploidy and its clinical and prognostic significance. The reported frequency of aneuploidy ranges from 28-83%, and up to 85% in untreated patients (San Miguel et al, 1995). The reasons for the discrepancies were evaluated in a DNA Cytometry Consensus Conference held in October 1992 (Duque et al, 1993), and are mentioned in Chapter 1.

In the present study, DNA ploidy was performed in 103 patients at presentation. 65 patients (63.1%) had a diploid pattern, while 38 patients (36.9%) demonstrated aneuploidy (35% hyperdiploidy and 1.9% hypodiploidy). Of the aneuploid population, the vast majority had hyperdiploidy (36/38 - 95%) and only a small minority had hypodiploidy (2/38 - 5%) (see Figures 3.10 and 3.11). The percentage of patients with aneupolidy is approximately 15-20% lower in our patients compared to most other series. The reasons for this finding are not entirely clear as the mean bone marrow plasma cell numbers at flow cytometry were 23.1%, suggesting a reasonably high abnormal population of cells. It is interesting to note that the mean bone marrow plasma cell percentage at morphology in the same group of patients is 35.3%. It is likely that plasma cells are lost during the preparation of the specimens (e.g. during ficoll separation) accounting for the lower number observed at flow cytometry.

In a large series of 156 untreated myeloma patients, aneuploidy was present in 58% of patients, with 56% hyperdiploidy and 2% hypodiploidy respectively (Garcia Sanz et al, 1995). Table 1.5 shows the results of 14 studies in which the incidence of DNA ploidy

was analysed by flow cytometry (San Miguel et al, 1995). Our findings are similar to these studies with respect to the dominant pattern of aneuploidy being hyperdiploidy. Hypodiploidy is uncommon, occurring at a frequency of 0 -15%. In some studies, hypodiploidy has been associated with a shorter survival and poor response to therapy (Morgan et al, 1989; Barlogie et al, 1985). A high incidence of hypodiploidy has been reported for plasma cell leukaemia (Shimazaki et al, 1992). There were only two patients with hypodiplody in our series. The first patient was a 61 year old female and the second a 75 year old male. Interestingly, both patients had stage 3A disease, IgA κ myeloma and manifested with central nervous system plasmacytomas at presentation. The first patient displayed an abnormal cytogenetic profile. Of the 10 metaphases that were obtained for analysis, 8 had a normal karyotype of 46,XX, 1 had a karyotype of 46,XX,del(10)(q23) and 1 46,XX,del(9),17q+. This patient is alive 36 months after diagnosis, and responding to treatment, while the second patient died 4 months after diagnosis. Cytogenetics were unsuccesssful in him. None of the patients had a plasma cell leukaemia.

In Table 3.41, a comparison between the different variables in the diploid and aneuploid groups is shown. The mean age was 63.3 years in the diploid group and 62.1 years in the aneuploid group. The mean bone marrow plasma cell numbers were statistically significantly higher (p=0.0005) in the aneuploid group, as were the serum paraprotein levels (p=0.0078) and IgG levels (particularly when the IgG level was >50 g/l - p=0.0082 and >70 g/l - p=0.0316). Abnormal cytogenetics were present in 50% of the aneuploid group, while only 14.2% of patients in the diploid group had abnormal cytogenetics. There was no difference in performance status, stage of disease, type of myeloma (heavy

and light chain isotypes) and association with plasmacytomas in the two groups. Also, the overall survival in the two groups was not statistically significantly different (p=0.7911). The average survival in the diploid group was 26 months compared to 24 months in the aneuploid group.

In addition to the DNA ploidy, relevant plasma cell antigen expression was also studied. Initially, different antigen expression on plasma cells which were localised on the bit map were studied. The results were difficult to interpret because of background staining and non-specific binding. The technique was changed to localising plasma cells based on the strong or 'bright' CD38 (CD38+++) expression. Double staining studies were then performed looking for co-expression of CD38 with CD56, CD45, CD33 and CD10.

In Table 3.42, a summary of CD38 antigen co-expression with CD56, CD45, CD33 and CD10 is shown. Myelomatous plasma cells show a higher percentage expression (positivity) only with respect to CD56. Lack of expression on plasma cells is higher with CD45, CD33 and CD10. This is consistent with the bone marrow myeloma cell phenotype of CD56++ as reported by Harada et al (1993) and the view of Pellat-Deceunynck et al (1994), that malignancy is characterised by increased expression of CD56.

CD38-56 antigen co-expression in relation to prognostic factors is detailed in Table 3.43. The known parameters of prognostic significance as defined in the background patient population and the case-control study were not significantly different in those who expressed CD38-56 versus those who did not. The single notable exception was the stage of the disease, in which the difference was highly significant (p=0.0013). More

patients with CD38-56 expression had advanced (stage 3 disease). Although the survival was shorter in the CD38-56 positive group (24 months compared to 37 months in the negative group), the difference was not statistically significant (p=0.1599). A correlation between loss of CD56 expression and extramedullary spreading of malignant plasma cells in myeloma patients has been reported (Pellat-Deceunynck et al, 1995). We were unable to show this correlation. In fact, our findings suggest the opposite pattern. Lack of CD38-56 expression is associated with a lower risk of extramedullary myeloma, although the risk is not statistically significant (p=0.8632). Furthermore, lack of CD56 expression has also been noted with light chain disease and associated with a shorter survival (Van Camp et al, 1990). We were unable to confirm this finding as CD38-56 expression was present in 50% of our patients and absent in the remaining 50%.

In contrast to myeloma, patients with plasma cell leukaemia do not express high levels of CD56 (Pellat-Deceunynck et al, 1995). Of the two patients in our series with plasma cell leukaemia, CD38-56 expression was not found in the one patient and not done in the other patient.

A recent review shows that CD56 expression is constant over the course of the disease, and the distribution of myeloma plasma cells in peripheral blood and bone marrow correlates with CD56 expression (Rawstron et al, 1999).

CD45 reactivity has been reported to be associated with immature plasma cells (Hata et al, 1994), although the expression of the antigen has not been extensively studied (Huelin et al, 1988). 43.9% of the patients in our series showed CD38-45 antigen co-expression. Expression of the antigen on plasma cells was not associated with any prognostic factors

(see Table 3.44), nor did its presence confer any significant survival benefit (average survival 27 months in the positive group versus 26 months in the negative group, p=0.7491).

The expression of myeloid related antigens has been reported in 6-65% of myeloma patients (Ruiz-Argüelles and San Miguel, 1994; Grogan et al, 1989). Myeloid markers appear to occur more frequently in patients with immature morphology and the co-expression of two or more myeloid related antigens appears to confer a poor outcome (San Miguel et al, 1991; Grogan et al, 1989). CD38-33 antigen co-expression was noted in 28.3% of the patients (see Table 3.45). Its presence is neither related to prognosis (see Table 3.50) nor is it related to survival (average survival 29 months in the positive group compared to 28 months in the negative group, p=0.9500).

CD10 has been noted with a variable frequency of 10-60% in myelomatous plasma cells (Ruiz-Argüelles and San Miguel, 1994; San Miguel et al, 1991; Epstein et al, 1990; Warburton et al, 1989). While Durie and Grogan (1985), have related it to very aggressive disease (4 patients with CALLA positive myeloma had a median survival of 6 months, compared to the CALLA negative group with a median survival of 56 months), Epstein et al (1990), have suggested that CD10+ patients display a more favourable clinical course. CD38-10 co-expression was present in 9.6% of the patients (see Table 3.42). CD38-10 positivity did not correlate with any of the other prognostic factors (see Table 3.46). Those patients who demonstrated CD38-10 co-expression had a longer average survival of 38 months compared to 27 months in the negative patients. However, the difference was not of statistical significance (p=0.9468).

CHAPTER 5

5.0 CONCLUSION

This study reveals some interesting and important information regarding myeloma in southern African blacks. As the case-control study, HHV-8 study, HLA study and flow cytometry study have not been performed previously in the above population, and indeed in Africa, both the positive and negative findings are relevant.

Two general areas of concern relate to the following, viz., i) The case-control study is very broad-based, and as there were no prior studies of a similar nature, this study was designed to capture as much information as possible. Analysis of the findings will now allow us to focus on areas of interest and importance and to leave out those aspects which are clearly negative or unrelated, and ii) the patients used in this study were all from a single hospital, and larger numbers may be required to validate some of the significant findings, especially in relation to the HLA study. In order to enhance patient numbers collaborative studies should be performed. Notwithstanding these shortcomings, the thesis does provide new and additional information in the many avenues studied. Some of the pertinent findings will be detailed below.

The median age at presentation is approximately five years younger than in the Western world. The patients present late, with advanced stage disease. The prognostic factors are similar to that described in the literature. The median survival of 15.5 months is shorter than that described in the literature.

A significantly increased risk with agriculture (farming) was found in the myeloma cases. Radiation, smoking and alcohol were not associated with myeloma. Socioeconomic

factors do not influence myeloma risk. Immunization during childhood appears to be protective.

The exact association of KSHV/HHV-8 and myeloma remains unclear. At present there appears to be no increased association with HIV in our patients.

An additional HLA association not documented previously, but present in our patients is HLA B35. HLA B35 confers a relative risk of 11.82. A number of haplotypes were found to confer an increased risk of myeloma while A2,B42; A30,B70 were protective.

Aneuploidy was present in 36.9% of the patients. Hyperdiploidy was the dominant pattern - 95%, while hypodiploidy was present in only 5% of the aneuploid group. A higher mean plasma cell number, paraprotein level (particularly IgG level) and abnormal cytogenetics were noted to be of significance when comparing the findings in the aneuploid and diploid groups. CD38-56 expression was higher in the myelomatous plasma cells. CD38-56 expression also correlated significantly with advanced stage disease and a shorter survival.

5.1 FUTURE RECOMMENDATIONS

A. Further and more detailed analysis should be undertaken with regard to the positive associations found in the case-control study, particularly the role of agriculture (farming) and myeloma.

B. The role of KSHV/HHV-8 needs clarification. The study should continue as the prevalence of KSHV appears to be higher in South Africa than in the Western world. The positivity in 4[illegible] adherent cell cultures should be confirmed with

one or more additional primer sets, to exclude the possibility that a positive result is due to PCR contamination. Nested PCR of DNA obtained from tissues not involved by myeloma should also be performed. Ideally, adherent cell cultures from bone marrows obtained from patients with other types of lymphoproliferative disorders should be studied, to confirm the specificity of the association with myeloma.

C. The positive findings in the HLA study should be validated with larger numbers.

D. The role of other antigens expressed on plasma cells, such as CD20 should be studied.

E. Clinical and therapeutic correlations should be included, where possible.

F. Colloborative studies should be undertaken in the areas of interest.

In many respects, the studies that constitute this thesis should be viewed as 'pilot' studies. It is hoped that these studies will provide the foundation on which more specific aspects of the epidemiology of myeloma in southern Africa will be studied.

REFERENCES

Acute Leukemia Group B. Correlation of abnormal immunoglobulin with clinical features of myeloma. Arch Intern Med 135, 46-52, 1975.

Agbalika F., Mariette X., Marolleau J.P., Fermand J.P. and Brouet J.C. Detection of human herpesvirus-8 DNA in bone marrow biopsies from patients with multiple myeloma and Waldenstrom's macroglobulinemia. Blood 91, 4393-4, 1998.

Aksoy M., Erdem S., Dincol G., Kutlar A., Bakioglu I. and Hepyuksel T. Clinical observations showing the role of some factors in the etiology of multiple myeloma. A study of 7 patients. Acta Haematol 71, 116-20, 1984.

Alavanja M.C.R., Blair A., Merkle S., Teske J. and Eaton B. Mortality among agricultural extension agents. Am J Ind Med 14, 167-76, 1988.

Alsina M., Gao S.J., Montalvo E.A., Leach C.T., Roodman G.D. and Jenson H.B. Increased prevalence of antibodies to Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in patients with multiple myeloma. Blood 90, 87a, 1997(suppl 1).

Ambroziak J.A., Blackbourn D.J., Herndier B.G., Glogau R.G., Gullett J.H., McDonald A.R. et al. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. Science 268, 582-3, 1995.

Ames B.N., Kammen H.O. and Yamashi E. Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. Proc Natl Acad Sci USA 72, 2423, 1975.

Anderson K.C., Park E.K., Bates M.P., Leonard R.C.F., Hardy R., Schlossman S.F. and Nadler L.M. Antigens on human plasma cells identified by monoclonal antibodies. J Immunol 130, 1132-8, 1983.

Andjelkovich D., Taulbee J. and Blum S. Mortality of female workers in a rubber manufacturing plant. J Occup Med 20, 409, 1978.

Axelsson U. A 20 year follow-up study of 64 patients with M-components. Acta Med Scand 219, 519, 1986.

Axelsson U., Bachmann R and Hällén J. Frequency of pathological proteins (M-components) in 6 995 sera from an adult population. Acta Med Scand 179, 235, 1966.

Avet-Loiseau H., Li J.Y., Godon C., Morineau N., Daviet A., Harousseau J.L. et al. P53 deletion is not a frequent event in multiple myeloma. Br J Haematol 106, 717-9, 1999.

Avet-Loiseau H., Li J.Y., Facon T., Brigadeau C., Morineau N., Maloisel F. et al. High incidence of translocations t(11;14)(q13;q32) and t(4;14)(p16;q32) in patients with plasma cell malignancies. Cancer Res 58, 5640-5, 1998.

Bakkus M.H.C., Van Riet I., Van Camp B. and Thielemans K. Evidence that the clonogenic cell in multiple myeloma originates from the pre-switched but somatically mutated B cell. Br J Haematol 87, 68-74, 1994.

Bancherau J., De Paoli P., Vallè A., Garcia E. and Rousset F. Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. Science 251, 70-4, 1991.

Barille S., Collette M., Bataille R. and Amiot M. Myeloma cells upregulate interleukin-6 secretion in osteoblastic cells through cell-to-cell contact but downregulate osteocalcin. Blood 86, 3151-9, 1995.

Barker H.F., Ball J., Drew M., Hamilton M.S., Franklin I.M. The role of adhesion molecules in multiple myeloma. Leuk Lymphoma 81, 331-5, 1992.

Bartl R., Frisch B. and Wilmanns W. Morphology of multiple myeloma. In Myeloma: Biology and Management. Eds. J.S. Malpas, D.E. Bergsagel and R.A. Kyle (1st ed.). Oxford University Press Inc., New York, 1995, p.82-123

Barlogie B. and Epstein J. Multiple myeloma: biology and therapy. Journal of Cancer Research and Clinical Oncology 116, 109-11, 1990.

Barlogie B., Epstein J., Selvanayagam P. and Alexanian R. Plasma cell myeloma. New biological insights and advances in therapy. Blood 73, 865-79, 1989.

Barlogie B., Alexanian R., Dixon D., Smith L., Smallwood L. and Delasalle K. Prognostic implications of tumor cell DNA and RNA content in multiple myeloma. Blood 66, 338-41, 1985.

Bataille R., Boccadoro M., Klein B., Durie B., and Pileri A. C-reactive protein and serum beta-2 microglobulin produce a simple and powerful myeloma staging system. Blood 80, 733-7, 1992a.

Bataille R., Chappard D. and Klein B. Mechanisms of bone lesions in multiple myeloma. Hematol Oncol Clin North Am. Ed. B. Barlogie. Philadelphia, PA: WB Saunders, 1992b, p.285-95.

Bataille R., Jourdan M., Zhang X.G. and Klein B. Serum levels of interleukin-6, a potent myeloma cell growth factor, as a reflection of disease severity in plasma cell dyscrasias. J Clin Invest 84, 2008-11, 1989.

Bataille R. and Grenier J. Serum beta-2 microglobulin in multiple myeloma, A critical review. Eur J Cancer Clin Oncol 23, 1829-32, 1987.

Beijersbergen R.L., Hijmans E.M., Zhu L. and Bernards R. Interaction of c-Myc with the pRb-related protein p107 results in the inhibition of c-Myc-mediated transactivation. EMBO J 13, 4080-6, 1994.

Benchaib M., Souchier C., Ffrench M., Remy F. and Bryon P.A. Fluorescence image analysis of plasma cells in monoclonal gammopathies. Anal Quant Cytol Histol 16, 137-44, 1994.

Bender A.P., Parker D.L., Johnson R.A., Scharber W.K., Williams A.N., Marbury M.C. and Mandel J.S. Minnesota highway maintenance worker study : Cancer mortality. Am J Ind Med 15, 545-56, 1989.

Beral V., Inskip H., Fraser P., Booth M., Coleman D. and Rose G. Mortality of employees of the United Kingdom Atomic Energy Authority, 1946-1979. BMJ 291, 440, 1985.

Berg J.W. and Burbank F. Correlations between carcinogenic trace metals in water supplies and cancer mortality. Ann NY Acad Sci 199, 249, 1972.

Bergamo A., Bataille R. and Pellat-Deceunynck P. CD40 and CD95 induce programmed cell death in the human myeloma cell line XG-2. Br J Haematol 97, 652-5, 1997.

Bergsagel D.E., Wong O., Bergsagel P.L., Alexanian R., Anderson K., Kyle R.A. et al. Benzene and Multiple Myeloma: Appraisal of the Scientific Evidence. Blood 94, 1174-82, 1999.

Bergsagel D.E. The role of chemotherapy in the treatment of multiple myeloma. In Bailliere's Clinical Haematology. Multiple Myeloma. Ed. F. Mandelli. Bailliere Tindall. 8(4), 1995, p.783-94.

Bergsagel P.L., Chesi M. and Kuehl W.M. Myeloma - a multistep transformation process. Abstract. VIIth International Multiple Myeloma Workshop, Sweden, 1999.

Bergsagel P.L., Chesi M., Nardini E., Brents L.A., Kirby S.L. and Kuehl W.M. Promiscuous translocations into immunoglobulin heavy chain switch regions in multiple myeloma. Proc Natl Acad Sci USA 93, 13931, 1996.

Bertrams J., Kuwert E., Böhme U., Reis H.E., Gallmeier W.M., Wetter O. et al. HLA antigens in Hodgkin's disease and multiple myeloma - increased frequency of W18 in both diseases. Tissue Antigens 2, 41, 1972.

Bethwaite P.B., Pearce N. and Fraser J. Cancer risks in painters: study based on the New Zealand Cancer Registry. Br J Ind Med 47, 742-6, 1990.

Bezabeh S., Engel A., Morris C.B. and Lamm S.H. Does benzene cause multiple myeloma? An analysis of the published case-control literature. Environ Health Perspect 104(6), 1393-8, 1996.

Billadeau D., Quam L., Thomas W., Kay N., Greipp P., Kyle R. et al. Detection and quantitation of malignant cells in the peripheral blood of multiple myeloma patients. Blood 80, 1818-24, 1992.

Birx D.L., Redfield R.R. and Tosato G. Defective regulation of Epstein-Barr virus infection in patients with the acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. N Engl J Med 314, 874-9, 1986.

Bladé J., Samson D., Reece D., Appperley J., Björkstrand B., Gahrton G. et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Br J Haematol 102, 1115-23, 1998.

Bladé J. and Kyle R.A. Monoclonal gammopathies of undetermined significance. In Myeloma: Biology and Management. Eds. J.S. Malpas, D.E. Bergsagel and R.A. Kyle. (1st ed.). Oxford University of Press Inc., New York, 1995, p.433-62.

Bladé J., López-Guillermo A., Rozman C., Cervantes F., Salgado C., Aguilar J.L. et al. Malignant transformation and life expectancy in monoclonal gammopathy of undetermined significance. Br J Haematol 81, 391, 1992.

Bladé J., Rozman C., Cervantes F., Reverter J.C. and Montserrat E. A new prognostic system for multiple myeloma based on easily available parameters. Br J Haematol 72, 507-11, 1989.

Blair A. and Zahm S.H. Herbicides and cancer. A review and discussion of methodologic issues. Recent Results Cancer Res 120, 132, 1990.

Blair A., Malker H., Cantor K.P., Burmeister L. and Wiklund K. Cancer among farmers. Scand J Work, Environ Health 11, 397-407, 1985.

Blattner W.A., Blair A. and Mason T.J. Multiple Myeloma in the United States, 1950-1975. Cancer 48, 2547-54, 1981.

Blattner W.A., Mason T.J. and Blair A. Changes in mortality rates from multiple myeloma. N Engl J Med 302, 814-5, 1980a.

Blattner W.A., Garber J.E., Mann D.L., McKeen E., Henson R., McGuire D.B. et al. Waldenstrom's macroglobulinemia and autoimmune disease in a family. Ann Intern Med 93, 830-2, 1980b.

Blattner W.A., Jacobson R.J. and Shulman G. Multiple myeloma in South African blacks. Lancet 1, 928, 1979.

Boffetta P., Stellman S.D. and Garfinkel L. A case-control study of multiple myeloma nested in the American Cancer Society prospective study. Int J Cancer 43, 554, 1989.

Boice J.D., Morin M.M., Glass A.G., Friedman G.D., Stovall M., Hoover R.N. et al. Diagnostic X-ray procedures and risk of leukemia, lymphoma and multiple myeloma. JAMA 265, 1290-4, 1991.

Boice J.D., Engholm G., Kleinerman R.A., Blettner M., Stovall M., Lisco H. et al. Radiation dose and second cancer risk in patients treated for cancer of the cervix. Radiat Res 116, 3-55, 1988.

Boice J.D., Day N.E., Andersen A., Brinton L.A., Brown R., Choi N.W. et al. Second cancers following radiation treatment for cervical cancer. An international collaboration among cancer registries. J Natl Cancer Inst 74, 955-75, 1985.

Boise L.H., Gonzalez-Garcia M., Postema C.E., Ding L., Lindsten T., Turka L.A. et al. Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74, 597-608, 1993.

Boshoff C., Schulz T.F., Kennedy M.M., Graham A.K., Fisher C., Thomas A. et al. Kaposi's sarcoma-associated herpesvirus infects endothelial cells and spindle cells. Nature Med 1, 1274-8, 1995.

Bourboulia D., Whitby D., Boshoff C., Newton R., Beral V., Carrara H. et al. Serologic evidence for mother-to-child transmission of Kaposi's sarcoma-associated herpesvirus infection. JAMA 280, 31-2, 1998.

Bourguet C.C., Grufferman S., Delzell E., Delong E.R. and Cohen H.J. Multiple myeloma and family history of cancer. Cancer 56, 2133-9, 1985.

Bourne H.R., Sanders D.A. and McCormick F. The GTP-ase superfamily : Conserved structure and molecular mechanism. Nature 349, 117, 1991.

Bowden M., Crawford J., Cohen H.J. and Noyatha O. A comparative study of monoclonal gammopathy and immunoglobulin levels in Japanese and United States elderly. J Am Geriatr Soc 41, 11-14, 1993.

Brenning G., Simonsson B., Kallander C. and Ahre A. Pretreatment serum β-2 microglobulin in multiple myeloma. Br J Haematol 62, 85-93, 1986.

Brousset P., Meggetto F., Attal M. and Delsol G. Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. Science 278, 1972, 1997.

Brown L.M., Burmeister L.F., Everett G.D. and Blair A. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes and Control 4, 153-6, 1993.

Brown L.M., Everett G.D., Gibson R., Burmeister L.F., Schuman L.M. and Blair A. Smoking and risk of non-Hodgkin's lymphoma and multiple myeloma. Cancer Causes and Control 3, 49-55, 1992.

Brown R.D., Pope B., Luo X-F., Gibson J. and Joshua D. The oncoprotein phenotype of plasma cells from patients with multiple myeloma. Leuk Lymphoma 16, 147, 1994.

Brownson R.C. Cigarette smoking and risk of myeloma. J Natl Cancer Inst 83, 1036, 1991.

Brownson R.C., Reif J.S., Chang J.C and Davis J.R. Cancer risk among Missouri farmers. Cancer 64, 238, 1989.

Brownson R.C. and Reiff J.S. A cancer registry-based study of occupational risk for lymphoma, multiple myeloma and leukemia. Int J Epidemiol 17, 27-32, 1988.

Brugiatelli M., Comis M., Iacopino P., Morabito F., Nobile V., Lombardo T. et al. Multiple myeloma in husband and wife. Acta Haematol 64, 227-9, 1980.

Bunn P.A., Krasnow S., Makuch R.W., Schlam M.L. and Schechter G.P. Flow cytometric analysis of DNA content of bone marrow cells in patients with plasma cell myeloma: clinical implications. Blood 59, 528-35, 1982.

Burger R., Neipel F., Fleckenstein B., Savino R., Ciliberto G., Kalden J.R. et al. Human herpesvirus type 8 interleukin-6 homologue is functionally active on human myeloma cells. Blood 91, 1858-63, 1998.

Burmeister L.F. Cancer in Iowa farmers: Recent results. Am J Ind Med 18, 295, 1990.

Burmeister L.F., Everett G.D., Van Lier S.F. and Isacson P. Selected cancer mortality and farm practices in Iowa. Am J Epidemiol 118, 72-7, 1983.

Calasanz M.J Cigudosa J.C., Odero M.D., Garcia-Foncillas J., Martin J., Ardanaz M.T. et al. Hypodiploidy and 22q11 rearrangements at diagnosis are associated with poor prognosis in patients with multiple myeloma. Br J Haematol 98, 418-25, 1997.

Cancer Registry of South Africa. Annual Report 1987. Publication of the Cancer Registry of South Africa. Ed. J. Metz. S.A. Institute for Medical Research, Johannesburg, 1987.

Cantor K.P. and Blair A. Farming and mortality from multiple myeloma: A case-control study with the use of death certificates. J Natl Cancer Inst 72, 251-5, 1984.

Carbone D.P., Koroo A.M.C., Linnonoila R.I., Jewett P. and Gazdar A.F. Neural cell adhesion molecule expression and messenger RNA splicing patterns in lung cancer cell lines are correlated with neuroendocrine phenotype and growth morphology. Cancer Res 51, 6142-9, 1991.

Carter A., Merchar S., Silvian-Draxler I. and Tatarsky I. The role of interleukin-1 and tumour necrosis factor-α in human multiple myeloma. Br J Haematol 74, 424-31, 1990.

Carter A. and Tatarsky I. The physiopathological significance of benign monoclonal gammopathy: a study of 64 cases. Br J Haematol 46, 565, 1980.

Cathomas G., Regamey N., Stalder A., Kurrer M.O., Joller-Jemelka H.I. and Erb P. Multiple myeloma and HHV-8 infection. Blood 91, 4391, 1998.

Catlett-Falcone R., Landowski T.H., Oshiro M.M., Turkson J., Levitzki A., Savino R. et al. Constitutive activation of STAT3 signalling confers resistance to apoptosis in human myeloma tumour cells. Immunity 10, 105-15, 1999.

Cesarman E., Chang Y., Moore P.S., Said J.W. and Knowles D.M. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body cavity-based lymphomas. N Engl J Med 332, 1186-91, 1995.

Chang Y., Cesarman E., Pessin M.S., Lee F., Culpepper J. and Knowles D.M. et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266, 1865-9, 1994.

Chesi M., Nardini E., Lim R.S., Smith K.D., Kuehl W.M. and Bergsagel P.L. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood 92, 3025, 1998.

Committee of Chronic Leukemia-Myeloma Task Force, National Cancer Institute: Proposed Guidelines for Protocol Studies. II Plasma Cell Myeloma. Cancer Chemother Rep 4, 145-58, 1973.

Clayberger C., Medeiros L.J., Link M.P., Warnke R.A., Wright A. and Koller T.D. et al. Absence of cell surface LFA-1 as a mechanism of escape from immunosurveillance. Lancet 2, 533-6, 1987.

Cohen H.J., Bernstein R.J. and Grufferman S. Role of immune stimulation in the etiology of multiple myeloma : a case-control study. Am J Hematol 24, 119-26, 1987.

Commotti B., Bassan R., Buzetti M., Finazzi G. and Barbui T. Multiple myeloma in a pair of twins. Br J Haematol 65, 123-4, 1987.

Cook G., MacKenzie J., Sheldon J., Moran G., Schulz T.F., Jarrett R.F. et al. Seroprevalence of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in patients with multiple myeloma is similar to healthy controls. Blood 90, 588a,1997(suppl 1).

Cook-Mozzaffari P.J., Darby S.C., Doll R., Forman D., Hermon C., Pike M.C. and Vincent T. Geographical variation of mortality from leukemia and other cancers in England and Wales in relation to proximity to nuclear installations, 1969-78. Br J Cancer 59, 476, 1989.

Corradini P., Inghirami G., Astolfi M., Ladetto M., Voena C., Ballerini P. et al. Inactivation of tumour suppressor genes, p53 and Rb1, in plasma cell dyscrasias. Leukemia 8, 758, 1994.

Corradini P., Ladetto M., Voena C., Palumbo A., Inghirami G., Knowles D.M. et al. Mutational activation of N- and K-ras oncogenes in plasma cell dyscrasias. Blood 81, 2708, 1993.

Crapper R.M., Deam D.R. and Mackay I. Paraproteinemias in homosexual men with HIV infection : lack of association with abnormal clinical or immunologic findings. Am J Clin Pathol 88, 348-51, 1987.

Crawford J. Eye M.K. and Cohen H.J. Evaluation of monoclonal gammopathies in the "well" elderly. Am J Med 82, 39, 1987.

Cull G.M., Timms J.M., Haynes A.P., Russel N.H., Irving W.L., Ball J.K. et al. Dendritic cells cultured from mononuclear cells and CD34 cells in myeloma do not harbour human herpesvirus 8. Br J Haematol 100, 793, 1998.

Cuzick J. Multiple Myeloma. Cancer Surveys 19-20, 455-74, 1994.

Cuzick J. International time trends for multiple myeloma. Ann NY Acad Sci 609, 205-14, 1990.

Cuzick J. and De Stavola B. Multiple myeloma - a case control study. Br J Cancer 57, 516-20, 1988.

Cuzick J., Velez R. and Doll R. International variations and temporal trends in mortality from multiple myeloma. Int J Cancer 32, 13-19, 1983.

Dancescu M., Wu C., Rubio M., Delespesse G. and Sarfati M. IL-4 induces conformational change of CD20 antigen via protein kinase C-independent pathway: antagonistic effect of anti-CD40 monoclonal antibody. J Immunol 148, 2411-6, 1992.

Danova M., Riccardi A., Ucci G., Luoni R., Giordano M. and Mazzini G. Ras oncogene expression and DNA content in plasma cell dyscrasias: a flow cytofluorimetric study. Br J Cancer 62, 781-5, 1990.

Dao D.D., Sawyer J.R., Epstein J., Hoover R.G., Barlogie B. and Tricot G. Deletion of the retinoblastoma gene in multiple myeloma. Leukemia 8, 1280-4, 1994.

Darby S.C., Kendall G.M., Fell T.P., Goodill A.A., Conquest A.J., Jackson D.A. et al. Further follow up of mortality and incidence of cancer in men from the United Kingdom who participated in the United Kingdom's atmospheric nuclear weapon tests and experimental programmes. BMJ 307, 1530-5, 1993.

Darby S.C., Kendall G.M., Fell T.P., O'Hagan J.A., Muirhead C.R., Ennis J.R. et al. A summary of mortality and incidence of cancer in men from the United Kingdom who participated in the United Kingdom's atmospheric nuclear weapon tests and experimental programmes. BMJ 296, 332-8, 1988.

Darby S.C., Doll R., Gill S.K. and Smith P.G. Long-term mortality after a single treatment course with X-rays in patients treated for ankylosing spondylitis. Br J Cancer 55, 179-90, 1987.

Davis D.L., Hoel D., Fox J. and Lopez A. International trends in cancer mortality in France, West Germany Italy, Japan, England and Wales and the USA. Lancet 2, 474, 1990.

Davis F.G. Boice J.D. Jr., Hrubec Z. and Monson R.R. Cancer mortality in radiation - exposed cohort of Massachusetts tuberculosis patients. Cancer Res 49, 6130-6, 1989.

Decouflé P., Blattner W.A. and Blair A. Mortality among chemical workers exposed to benzene and other agents. Environ Res 30, 15, 1983.

DeGreef C., Bakkus M., Heirman C., Schots R., Lacor P., De Waele M. et al. The absence of Kaposi's sarcoma-associated herpesvirus (KSHV) DNA sequences in leukapheresis products and ex-vivo expanded $CD34^+$ cells in multiple myeloma (MM) patients. Blood 90, 86a, 1997(suppl 1).

Delzell E. and Grufferman S. Cancer and other causes of death among female textile workers, 1976-78. J Natl Cancer Inst 71, 735, 1983.

De Martinis M., Ginaldi L., D'Ostilio A., Marini L., Di Gennaro A. and Quaranino D. Simultaneous phenotypic and kinetic analysis in multiple myeloma. Blood 84, 652a, 1995(suppl 1).

Demers P.A., Vaughan T.L., Koepsell T.D., Lyon J.L., Swanson G.M., Greenberg R.S. et al. A case-control study of multiple myeloma and occupation. Am J Ind Med 23, 629-39, 1993.

Devesa S.S. Descriptive epidemiology of multiple myeloma. In Epidemiology and Biology of Multiple Myeloma. Eds. G.I. Obrams and M. Potter. Springer-Verlag, Berlin, 1991, p.3-12.

Devesa S.S., Silverman D.T., Young J.L., Pollack E.S., Brown C.C., Horm J.W. et al. Cancer incidence and mortality trends among Whites in the United States, 1947-84. J Natl Cancer Inst 79, 701-70, 1987.

Dewald G.W. and Jenkins R.B. Cytogenetic and molecular genetic studies of patients with monoclonal gammopathies. In Neoplastic diseases of the blood. Eds. P.H. Wiernik, G.P. Canellos, R.A. Kyle and C.A. Schiffer. (2nd ed.). Churchill Livingstone, New York, 1991, p.427.

Dewald G.W., Kyle R.A., Hicks G.A. and Greipp P.R. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. Blood 66, 380-90, 1985.

Divine B.J. and Barron V. Texaco mortality study : III. A cohort of producing and pipeline workers. Am J Ind Med 11, 189, 1987.

Divine B.J., Barron V. and Kaplan S.D. Texaco mortality study : I. Mortality among refinery, petrochemical and research workers. J Occup Med 27, 445, 1985.

Dodson M.K., Cliby W.A., Xu H-J., De Lacey K.A. Hu S-X., Keeney G.L. et al. Evidence of functional Rb protein in epithelial ovarian carcinomas despite loss of heterozygosity at the Rb locus. Cancer Res 54, 610, 1994.

Dolphin G.W. A comparison of the observed and expected cancers of the haematopoietic and lymphatic systems among workers at Windscale: A first report. Harwell, England, National Radiation Protection Board NRPB-R54, 1976.

Dousset M. Cancer mortality around La Hague nuclear facilities. Health Phys 56, 875, 1989.

Downward J. Regulatory mechanisms for ras proteins. Bioassays 14, 177, 1992.

Drach J., Schuster J., Nowotny H., Angerler J., Rosenthal F., Fiegl M. et al. Multiple myeloma : High incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. Cancer Res 55, 3854, 1995.

Drewinko B., Alexanian R., Boyer H., Barlogie B. and Rubinow S.I. The growth fraction of human myeloma cells. Blood 57, 333, 1981.

Du Toit E.D., MacGregor K.J., Taljaard D.G. and Oudshoorn M. HLA-A, B, C, DR and DQ polymorphisms in three South African population groups: South African Negroes, Cape Coloureds and South African Caucasoids. Tissue Antigens 31, 109-25, 1988.

Dubois B., Vanbervleit B., Fayette J., Masscrier C., Van Kooten C., Briere F. et al. Dendritic cells enhance growth and differentiation of CD40-activated B lymphocytes. J Exp Med 185, 941, 1997.

Dubrow R. and Gute D.M. Cause-specific mortality among male textile workers in Rhode Island. Am J Ind Med 13, 439, 1988.

Dunn T.B. Plasma cell neoplasms beginning in the ileocaecal area in strain C_3H mice. J Natl Cancer Inst 19, 371, 1957.

Dupin N., Fisher C., Kellam P., Ariad S., Tulliez M., Franck N. et al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci USA 96, 4546-51, 1999.

Dupin N., Grandadam M., Calvez V., Gorin I., Aubin J.T., Havard S. et al. Herpes-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. Lancet 345, 761-2, 1995.

Duque R.E., Andreef M., Braylan R.C., Diamond L.W. and Peiper S.C. Consensus review of the clinical utility of DNA flow cytometry in neoplastic hematopathology. Cytometry 14, 492-6, 1993.

Durie B.G.M. Cellular and molecular genetic features of myeloma and related disorders. Haematol Oncol Clin North Am 6, 463-77, 1992.

Durie B.G.M., Vela E.E. and Baum V. Cytogenetic abnormalities in multiple myeloma. In Epidemiology and biology of multiple myeloma. Eds. G.I. Obrams and M. Potter. Springer-Verlag, New York, 1991, p.137-41.

Durie B.G.M., Stock-Novak D., Salmon S.E., Finley J., Beckord J., Crowley J. et al. Prognostic value of pretreatment serum β2 microglobulin in myeloma: a south-west oncology group study. Blood 125, 823-30, 1990.

Durie B.G.M. Staging and kinetics of multiple myeloma. Semin Oncol 13, 300-9, 1986.

Durie B.G.M. and Grogan T.M. CALLA-positive myeloma: an aggressive subtype with poor prognosis. Blood 66, 229-32, 1985.

Durie B.G.M. and Salmon S.E. A clinical staging system for multiple myeloma. Cancer 36, 842-54, 1975.

Edwards A.W.F. and Cavalli-Sforza L.L. Affinity as revealed by differences in gene frequencies. In The assessment of population affinities in man. Eds. J.S. Weiner and J. Huizinga. Oxford University Press, Oxford, 1972, p.37-47.

Egedahl R.D., Coppock E. and Homik R. Mortality experience at a hydrometallurgical nickel refinery in Fort Saskatchewan, Alberta between 1954 and 1984. J Soc Occup Med 41, 29, 1991.

Epstein J., Hoover R., Kornbluth J. and Barlogie B. Biological aspects of multiple myeloma. In Multiple Myeloma. Ed. F. Mandelli. Bailliere's Clinical Haematology. London: Bailliere Tindall, 1995, p.721-34.

Epstein J., Xiao H. and He X.Y. Markers of multiple hematopoietic lineages in multiple myeloma. N Engl J Med 322, 644-68, 1990.

Eriksson M. Rheumatoid arthritis as a risk factor for multiple myeloma: a case-control study. Eur J Cancer 29A, 259-63, 1993.

Eriksson M. and Hallberg B. Familial occurrence of hematologic malignancies and other diseases in multiple myeloma: a case-control study. Cancer Causes and Control 3, 63-7, 1992.

Eriksson M. and Karlsson M. Occupational and other environment factors and multiple myeloma : a population-based case control study. Br J Ind Med 49, 95-103, 1992.

Ewen M.E., Sluss H.K., Sherr C.J., Matsushime H., Kato J., Livingston D. et al. Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. Cell 73, 487, 1993.

Farhangi M. and Osserman E.F. Biology, clinical patterns and treatment of multiple myeloma and related plasma cell dyscrasias. In The Immunopathology of Lymphoreticular Neoplasms, Comprehensive Immunology. Eds. J.J. Twomey and R.A. Good. Plenum Medical Book Company, New York, 1978.

Feinman R., Sawyer J., Hardin J. and Tricot G. Cytogenetics and Molecular Genetics in Multiple Myeloma. In Haematol Oncol Clin North Am. Ed. B. Barlogie.W.B. Saunders Company, Philadelphia, 1997, p.1-25.

Festen J.J.M., Marrink J., de Waard-Kuiper E.H. and Mandema E. Immuglobulins in families of myeloma patients. Scand J Immunol 6, 887-96, 1977.

Festen J.J.M., Marrink J., Sijpesteijn J.A.K., Loghem E.V., Nijenhuis L.E. and Mandema E. A study on the association between myelomatosis and immunoglobulin allotypes, HLA and blood groups. Immunogenetics 3, 201-3, 1976.

Fiedler W., Weh H.J. and Hossfeld D.K. Comparison of chromosome analysis and BCL-1 rearrangement in a series of patients with multiple myeloma. Br J Haematol 81, 58-61, 1992.

Figarella-Branger D.F., Durbec P.L. and Rougon G.N. Differential spectrum of expression of neural cell adhesion molecules isoforms and L1 adhesion molecules on human neuroectodermal tumors. Cancer Res 50, 6364-70, 1990.

Fine J.M., Lambin P. and Muller J.Y. The evolution of asymptomatic monoclonal gammopathies. A follow-up of 20 cases over periods of 3-14 years. Acta Med Scand 205, 339, 1979.

Flactif M., Lai J.L., Zandecki M., Bernardi F., Trillot N., Bauters F. et al. Two cases of translocation t(1;16)(p11;p11) in multiple myeloma: Confirmation by chromosome painting. Cancer Genet Cytogenet 76, 10, 1994.

Flodin U., Frederiksson M. and Persson B. Multiple myeloma and engine exhausts, fresh wood and creosote: a case referent study. Am J Ind Med 12, 519-20, 1987.

Forman D., Cook-Mozzaffari P., Darby S., Davey G., Stratton I., Doll R. and Pike M. Cancer near nuclear installations. Commentary. Nature 329, 499-505, 1987.

Friedman G.D. Cigarette smoking, leukemia and multiple myeloma. Annals of Epidemiology 3, 425-8, 1993.

Friedman G.D. Multiple myeloma : Relation to propoxyphene and other drugs, radiation and occupation. Int J Epidemiol 15, 424, 1986.

Gabrea A., Bergsagel P.L., Chesi M., Shou Y. and Kuehl W.M. Insertion of IgH switch sequences causes overexpression of cyclin D1 in a myeloma tumor cell. Mol Cell 3, 119, 1999.

Gahrton G.F., Zech L., Nilsson K., Lonnquist B. and Carlstrom A. Two translocations, t(11;4) and t(1;6) in a patient with plasma cell leukaemia and two populations of plasma cells. Scand J Haematol 24, 42-6, 1980.

Gaillard J.P., Bataille R., Brailly H., Zuber C., Yasukawa K., Attal M. et al. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gammopathy. Eur J Immunol 23, 820-4, 1993.

Gallagher R.P., Spinelli J.J., Elwood J.M. and Skippen D.H. Allergies and agricultural exposure and risk factors for multiple myeloma. Br J Cancer 48, 853, 1983.

Gallagher R.P. and Threfall W.J. Cancer mortality in metal workers. Can Med Assoc J 129, 1191, 1983.

Gao S.J., Kingsley L., Li M., Zheng W., Parravicini C., Ziegler J. et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nature Med 2, 925-8, 1996.

Garcia-Marco J., Caldas C., Price C.M., Wiedemann L.M., Ashworth A. and Catovssky D. Frequent somatic deletion of the 13q 12.3 locus encompassing BRCA2 in chronic lymphocytic leukemia. Blood 88, 1568, 1996.

García-Sanz R., Orfão A., González M., Moro M.J., Hernández J.M., Ortega F. et al. Prognostic implications of DNA aneuploidy in a series of 156 untreated myeloma patients. Br J Haematol 90, 106-12, 1995.

Gartner S. and Kaplan H. Long-term culture of human bone marrow cells. Proc Natl Acad Sci USA 77, 4756-9, 1980.

Gazdar A.F., Oie H.K., Kirsh I.R. and Hollis G. F. Establishment and characterization of a human plasma cell myeloma culture having a rearranged cellular myc proto-oncogene. Blood 67, 1542-9, 1986.

Goedert J.J, Cote T.R., Vigro P., Scoppa, Kingma D.W., Gail M.H. et al. Spectrum of AIDS-associated malignant disorders. Lancet 351, 1833-9, 1998.

Geschickter C.F. and Copeland M.M. Multiple myeloma. Arch Surg 16, 807-63, 1928.

Gilbert E.S., Fry S.A., Wiggs L.D., Voelz G.L. and Petersen G.R. Analyses of combined mortality data at the Hanford Site, Oak Ridge National Laboratory and Rocky Flats Nuclear Weapons Plant. Radiat Res 120, 19, 1989a.

Gilbert E.S., Petersen G.R. and Buchanan J.A. Mortality of workers at the Hanford Site: 1945-1981. Health Physics 56,11,1989b.

Giles G.G., Lickiss J.N., Baikie M.J., Lowenthal R.M. and Panton S. Myeloproliferative and lymphoproliferative disorders in Tasmania, 1972-1980 : Occupational and familial aspects. J Natl Cancer Inst 72, 1233,1984.

Glover C.S., Horm J. and Christenson G. Race and socioeconomic status differences in multiple myeloma survival. In Epidemiology and Biology of Multiple Myeloma. Eds. G.I. Obram and M. Potter. Berlin, Springer-Verlag, 1991, p.55-60.

Golden A.L., Markowitz S.B. and Landrigan P.J. The risk of cancer in firefighters. Occup Med 10(4), 803-20, 1995.

Goldenberg G.J., Parakevas F. and Israels L.G. The association of rheumatoid arthritis with plasma cell lymphocytic neoplasms. Arthritis Rheum 12, 569-79, 1969.

Goldstein B.D. Is exposure to benzene a cause of human multiple myeloma? Ann NY Acad Sci 609, 225, 1990.

Gonchoroff N.J., Katzmann J.A., Garton J.P., Ruiz-Arguelles G.J., Eilers C.R., Greipp P.R. and Kyle R.A. A monoclonal antibody reactive with a subset of human plasma cells. Br J Haematol 62, 619-30, 1986.

Gould J., Alexanian R., Goodacre A., Pathak S., Hecht B. and Barlogie B. Plasma cell karyotypes in multiple myeloma. Blood 71, 453-56, 1988.

Gramenzi A., Buttino I., D'Avanzo B., Negri E., Franceschi S. and La Vecchia C. Medical history and the risk of multiple myeloma. Br J Cancer 63, 769-72, 1991.

Greipp P.R., Lust J.A., O'Fallon W.M., Katzmann J.A., Witzig T.E. and Kyle R.A. Plasma cell labelling index and β2-microglobulin predict survival independent of thymidine kinase and C-reactive protein in multiple myeloma. Blood 81, 3382-7, 1993.

Grogan T.M., Durie B.G.M., Spier C.M., Richter L. and Vela E. Myelomonocytic antigen positive multiple myeloma. Blood 73, 763-9, 1989.

Groopman J.E., Sullivan J.L., Mulder C., Ginsburg D., Orkin S.H. O'Hara C.J. et al. Pathogenesis of B-cell lymphoma in a patient with AIDS. Blood 67, 612-5, 1986.

Grosbois B., Gueguen M., Fauchet R., Lebouc H., Guenot A., Lancelin F. et al. Multiple myeloma in two brothers. Cancer 58, 2417-21, 1986.

Grufferman S., Cohen H.J., Delzell E., Morrison M.C., Schold S.C. Jr. and Moore J.O. Familial aggregation of multiple myeloma and central nervous system diseases. J Am Geriatr Soc 37, 303-9, 1989.

Guidotti S., Wright W.E. and Peters J.M. Multiple myeloma in cosmetologists. Am J Ind Med 3, 169, 1982.

Gustavsson P., Gustavsson A. and Hogstedt C. Excess of cancer in Swedish chimney sweeps. Br J Ind Med 45, 777, 1988.

Gustavsson P., Hogstedt C. and Holmberg B. Mortality and incidence of cancer among Swedish rubber workers, 1952-1981. Scand J Work Environ Health 12, 538, 1986.

Haas H., Anders S., Bornkamm G.W., Mannweiler B., Schmitz H., Radl J. et al. Do infections induce monoclonal immunoglobulin components? Clin Exp Immunol 81, 435-40, 1990.

Haas-Kogan D.A., Kogan S.C., Levi D., Dazin P., T'Ang A., Fung Y-K.T. et al. Inhibition of apoptosis by the retinoblastoma gene product. EMBO J 14, 461-72, 1995.

Hagmar L., Bellander T., Englander V., Ranstam J., Attewell R. and Skerfving S. Mortality and morbidity among workers in a chemical factory. Scand J Work Environ Health 12, 545-51, 1986.

Hallek M., Bergsagel P.L. and Anderson K.C. Multiple Myeloma: Increasing evidence for a multistep transformation process. Blood 91, 3-21, 1998.

Hanis N.M., Shallenberger L.G., Donaleski D.L. and Sales E.A. A retrospective mortality study of workers in three major U.S. refineries and chemical plants. Part 1: Comparisons with U.S. population. J Occup Med 27, 283, 1985.

Hansen N.E., Karle H. and Olsen J.H. Trends in the incidence of multiple myeloma in Denmark 1943-1982: A study of 5500 patients. Eur J Haematol 42, 72-6, 1989.

Hansen J.A., Martin P.J. and Nowinski R.C. Monoclonal antibodies identifying a novel T cell antigen of human lymphocytes. Immunogenetics 10, 247-60, 1980.

Hara T., Fu S.M. and Hansen J.A. A new activation pathway used by a major T cell population via a disulfide-bonded dimer of a 44 kilodalton polypeptide (9.3 antigen). J Exp Med 161, 1513-24, 1985.

Harada H., Kawano M.M., Huang N., Harada Y., Iwato K., Tanabe O. et al. Phenotypic difference of normal plasma cell from mature myeloma cells. Blood 81, 2658-63, 1993.

Haralambindon S., Melo J.V. and Catowsky D. Different reactivity of monoclonal antibodies against common acute lymphoblastic leukemia antigen. J Clin Path 40, 490-3, 1987.

Hardin J., Macleod S., Grigorieva I., Chang R., Barlogie B., Xiao H. et al. Interleukin-6 prevents dexamethasone-induced myeloma cell death. Blood 84, 3063-70, 1994.

Harding F.A., McArthur J.G., Gross J.A., Raulet D.H. and Allison J.P. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T cell clones. Nature 356, 607-9, 1992.

Hata H., Matsuzaki H., Sonoki T., Takemoto S., Kuribaynshi N., Nagasaki A. and Takatsuki K. Establishment of a CD45-positive immature plasma cell line from an aggressive multiple myeloma with high serum lactate dehydrogenase. Leukemia 8, 1768-73, 1994.

Hateboer G., Marc Timmers H.T., Rustgi A.K., Billaud M., van 'T Veer L. and Bernards R. TATA-binding protein and the retinoblastoma gene protein and the retinoblastoma gene product bind to overlapping epitopes on c-myc and adenovirus E1A protein. Proc Natl Acad Sci USA 90, 8489-93, 1993.

Hayakawa N., Ohtaki M., Ueoka H., Matsuura M., Munaka M. and Kurihara M. Mortality statistics of major causes of death among atomic bomb survivors in Hiroshima Prefecture from 1968-1982. Hiroshima J Med Sci 38, 53-67, 1989.

Hayes R.B., Blair A., Stewart P.A., Herrick R.F. and Mahar H. Mortality of U.S. embalmers and funeral directors. Am J Ind Med 18, 641-652, 1990.

Heineman E.F., Olsen J.H., Pottern L.M., Gomez M., Raffu E. and Blair A. Occupational risk factors for multiple myeloma among Danish men. Cancer Causes and Control 3, 555-68, 1992a.

Heineman E.F., Zahm S.H., McLaughlin J.K., Vaught J.B. and Hrubec Z. A prospective study of tobacco use and multiple myeloma: evidence against an association. Cancer Cause and Control 3, 31-6, 1992b.

Helfrich M.H., Livingston E., Franklin I.M. and Soutar R.L. Expression of adhesion molecules in malignant plasma cells in multiple myeloma: comparison with normal plasma cells and functional significance. Blood Reviews 11, 28-38, 1997.

Heriot K., Hallquist A.E. and Tomar R.H. Paraproteinemia in patients with acquired immunodeficiency syndrome (AIDS) or lymphadenopathy syndrome (LAS). Clin Chem 31, 1224-6, 1985.

Herrinton L.J., Weiss N.S. and Olshan A.F. The epidemiology of myeloma. In Myeloma - Biology and . 'anagement. Eds. J.S. Malpas, D.E. Bergsagel and R.A. Kyle. (1st ed.). Oxford University Press, Inc., New York, 1995, p.127-68.

Herrinton L.J., Weiss N.S., Koepsell T.D., Daling J.R., Taylor J.W., Lyon J.L. et al. Exposure to hair-coloring products and the risk of multiple myeloma. Am J Public Health 84(7), 1142-4, 1994.

Herrinton L.J. Demers P.A., Koepsell T.D., Weiss N.S., Daling D.R., Taylor J.W. et al. Epidemiology of the M-component immunoglobulin types of multiple myeloma. Cancer Causes and Control 4(2), 83-92, 1993.

Herrinton L.J., Koepsell T.D. and Weiss N.S. Smoking and multiple myeloma. Cancer Causes and Control 3, 391-2, 1992.

Hewell G.M. and Alexanian R. Myeloma in young persons. Ann Intern Med 84, 441-3, 1976.

Holland J.F., Hosley H., Scharlau C., Carbone P.P., Frei E., Brindley C.O. et al. A controlled trial of urethane treatment in multiple myeloma. Blood 27, 328-42, 1966.

Hollstein M., Sidransky D., Vogelstein B., Halery O., Michalovitz D. and Oren M. Different Tumor-derived p53 mutants exhibit distinct biological properties. Science 250, 113-6, 1990.

Hoover R.N., Mason T.J., McKay F.W. and Fraumeni J.F. Geographic patterns of cancer mortality in the United States. In Persons at high risk of cancer, an approach to cancer etiology and control. Ed. J. Fraumeni. Academic Press, New York, 1975, p.343-60.

Horwitz L.J., Levy R.N., and Rosner F. Multiple myeloma in three siblings. Arch Intern Med 145, 1449-50, 1985.

Huang Y.Q., Li J.J., Kaplan M.H., Poiesz B., Katabira E., Zhang W.C. et al. Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. Lancet 345, 759-61, 1995.

Huelin C., Gonzalez M., Pedrinasi S., De La Higuera B., Piris M.A., San Miguel J. et al. Distribution of the CD45R antigen in the maturation of the lymphoid and myeloid series: the CD45R negative phenotype is a constant finding in T CD4 positive lymphoproliferative disorders. Br J Haematol 69, 173-9, 1988.

Hyjek E., Fata F., Niesvisky R., Michaeli J. and Cesarman E. Low prevalence of antibodies to Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in patients with multiple myeloma. Blood 90, 87a, 1997(suppl 1).

Ichimaru M., Ishimaru T., Mikami M. and Matsunaga M. Multiple myeloma among atomic bomb survivors in Hiroshima and Nagasaki, 1950-76: Relationship to radiation dose absorbed by marro v. J Natl Cancer Inst 69, 323-8, 1982.

Isomaki H.A., Hakulinen T. and Joutsenlahti U. Excess risk of lymphomas, leukemia and myeloma patients with rheumatoid arthritis. J Chron Dis 31, 691-6, 1978.

Jablon S., Hrubec Z., Boice J.D. Jr. and Stone B.J. Cancer in Populations Living Near Nuclear Facilities, Vol 1: Report and summary. (DHHS Publ (NIH) No 90-874). US Government Printing Office, Washington, DC, 1990.

Jackson N., Ling N.R., Ball J., Bromidge E., Nathan P.D. and Franklin I.M. An analysis of myeloma plasma cell phenotype using antibodies defined at the III international workshop on human leucocyte differentiation antigens. Clin Exp Immunol 72, 351-6, 1988.

Jacobson R.J. Multiple Myeloma in Sub-Sahara Africa. In Epidemiology and Biology of Multiple Myeloma. Eds. G.I. Obrams and M. Potter. Springer-Verlag, Berlin, 1991, p.69-71.

Jacobson R.J., Levy J.I., Shulman G. and De Moor N.G. Solitary Myeloma. A study of 10 black patients during an 8-year period. S Afr Med J 49, 1347-51, 1975.

Jancelewicz Z., Takatsuki K., Sugai S. and Pruzanski W. IgD multiple myeloma: review of 133 cases. Arch Intern Med 135, 87-93, 1975.

Jeannet M. and Magnin C. HLA-antigens in haematological maligant disease. Eur J Clin Invest 2, 39-42 1976.

Jensen G.S., Belch A.R., Mant M.J., Reuther B.A. and Pilarski L.M. Expression of multiple adhesion molecules on circulating monoclonal B cells in myeloma. Current Topics Microbiol Immunol 182, 187-93, 1991.

Jonhston J.M., Grufferman S., Bourguet C.C., Delzell E., de Long E.R. and Cohen H.J. Socioeconomic status and risk of multiple myeloma. J Epidemiol Community Health 39, 175, 1985.

Joshua D.E., Brown R.D. and Gibson J. Multiple myeloma : Why does the disease escape from plateau phase? Br J Haematol 88, 667-71, 1994.

Judson I.R., Wiltshaw E. and Newland A.C. Multiple Myeloma in a pair of monozygotic twins: the first reported case. Br J Haematol 60, 551, 1985.

Kapadia S.B. Multiple myeloma: a clinicopathologic study of 62 consecutively autopsied cases. Medicine (Baltimore) 59, 380-92, 1980.

Kaplan S.D. Update of a mortality study of workers in petroleum refineries. J Occup Med 28, 514, 1986.

Kardinal C.G. Multiple myeloma in a husband and wife. JAMA 239, 22, 1978.

Kato I., Tajima K., Hirose K., Nakagawa N., Kuroishi T. and Tominaga S. A descriptive epidemiological study of haematopoietic neoplasms in Japan. Japanese Journal of Clinical Oncology 15, 347-64, 1985.

Kawano M.M., Mihara K., Huang N., Tsujimoto T. and Kuramoto A. Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. Blood 85, 487-94, 1995.

Kawano M.M., Huang N., Harada H., Harada Y., Sakai A., Tanaka H. et al. Identification of immature and mature myeloma cells in the bone marrow of human myelomas. Blood 82, 564-70, 1993.

Kawano M., Hirano T., Matsuda M., Taga T., Horii Y., Iwato K. et al. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 332, 83-5, 1988.

Kedes D.H., Ganem D., Ameli N., Bachetti P. and Greenblatt R. The prevalence of serum antibody to human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) among HIV-seropositive and high-risk HIV-seronegative women. JAMA 277, 478-81, 1997.

Kedes D.H., Operskalski E., Busch M., Kohn R., Flood J. and Ganem D.E. The seroepidemiology of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nature Med 2, 918-24, 1996.

Keshava-Prasad H.S., Prangnell D.R. and Adelman M.I. Multiple myeloma in spouses. Clin Lab Haem 18, 61-4, 1996.

Khosravi-Far R. and Der C.J. The ras signal transduction pathway. Cancer Metastasis Rev 13, 67, 1994.

Klein B., Zhang X-G., Lu- Z-Y. and Bataille R. Interleukin-6 in multiple myeloma. Blood 85, 863-72, 1995.

Klein B., Zhang X-G., Jourdan M., Boiron J.M., Portier M., Lu Z.Y. et al. Interleukin-6 is the central tumour growth factor in vitro and in vivo in multiple myeloma. Eur Cytokine Network 1, 193, 1990.

Klein B., Zhang X-G., Jourdan M., Content J., Houssiau F., Aarden L. et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by IL-6. Blood 73, 517-26, 1989.

Koepsell T.D., Daling J.R., Weiss N.S., Taylor J.W., Olshan A.F., Lyon J.L. et al. Antigenic stimulation and the occurrence of multiple myeloma. Am J Epidemiol 126, 1051-62, 1987.

Koessel S.L., Theis M.K., Vaughan T.L., Koepsell T.D., Weiss N.S., Greenberg R.S. et al. Socioeconomic status and the incidence of multiple myeloma. Epidemiology 7(1), 4-8, 1996.

Konrad R.J., Kricka L.J., Goodman J. and Silberstein L.E. Myeloma-associated paraprotein directed against the HIV-1 p24 antigen in an HIV-seropositive patient. N Engl J Med 328, 1817-9, 1993.

Kornbluth J. Potential role of CD28-B7 interactions in the growth of myeloma plasma cells. Current Topics in Microbiology and Immunology 194, 43-9, 1995.

Korst D.R., Clifford G.O., Fowler W.M., Louis J., Will J. and Wilson H.E. Multiple myeloma. II. Analysis of cyclophosphamide therapy in 165 patients. JAMA 189, 156-61, 1964.

Korsmeyer S.J. Bcl-2 initiates a new category of oncogenes: Regulators of cell death. Blood 80, 879, 1992.

Kozbor D., Moretta A., Messner H.A., Moretta L. and Croce C.M. Tp44 molecules involved in antigen-independent T cell activation are expressed on human plasma cells. J Immunol 138, 4128-32, 1987.

Kumar S., Kumar D., Schnadig V.J., Selvanayagam P. and Slaughter D.P. Plasma cell myeloma in patients who are HIV-positive. Am J Clin Pathol 102(5), 633-9, 1994.

Kyle R.A. Monoclonal Gammopathy of Undertermined Significance and Solitary Plasmacytoma. Hematol Oncol Clin North Am 11, 71-87, 1997.

Kyle R.A. Monoclonal gammopathy of undetermined significance (MGUS). Bailliere's Clinical Haematology 8, 761-81, 1995.

Kyle R.A., Beard C.M., O'Fallon W.M. and Kurland L.T. Incidence of multiple myeloma in Olmsted County, Minnesota: 1978 through 1990, with a review of the trend since 1945. J Clin Oncol 12, 1577, 1994.

Kyle R.A. and Greipp P.R. Plasma cell dyscrasias: current status. CRC Critical Reviews in Oncology/Hematology 8, 93-152, 1988.

Kyle R.A. 'Benign' monoclonal gammopathy: a misnomer? JAMA 251, 1849-54, 1984.

Kyle R.A. and Greipp P.R. Multiple myeloma. Houses and spouses. Cancer 51, 735-9, 1983.

Kyle R.A. Multiple myeloma. Review of 869 cases. Mayo Clin Proc 50, 29-40, 1975.

Kyle R.A., Finkelstein S., Elveback L.R. and Kurland L.T. Incidence of monoclonal proteins in a Minnesota commmunity with a cluster of multiple myeloma. Blood 40, 719-24, 1972.

Kyle R.A., Heath C.W. Jr. and Carbone P. Multiple Myeloma in Spouses. Arch Intern Med 127, 944-6, 1971.

Kyle R.A., Nobrega F.T. and Kurland L.T. Multiple myeloma in Olmsted County, Minnesota, 1945-1964. Blood 33, 739-45, 1969.

La Vecchia C. and Tavani A. Epidemiological evidence on hair dyes and the risk of cancer in humans. Eur J Cancer Prev 4(1), 31-43, 1995.

La Vecchia C., Negri E., D'Avanzo B. and Franceshi S. Occupation and lymphoid neoplasms. Br J Cancer 60, 385, 1989.

Lai J., Zandecki M., Mary J.Y., Bernardi F., Izydorczyk V., Flactif M. et al. Improved cytogenetics in multiple myeloma : A study of 151 patients including 117 patients at diagnosis. Blood 85, 2490-7, 1995.

Lane H.C., Masur H., Edgar L.C., Whalen L.G., Rood A.H. and Fauci A.S. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med 309, 453-8, 1983.

Lanier L.L. and Hemperley J.J. CD56 and CD57 cluster workshop report. In Leucocyte Typing V. Ed. S.F. Schlossman. Oxford University Press, 1995, p.1398-400.

Latreille J. Barlogie B., Johnston D., Drewinko B. and Alexanian R. Ploidy and proliferative characteristics in monoclonal gammopathies. Blood 59, 43-51, 1982.

Latreille J., Barlogie B., Dosik G., Johnston D.A., Drewinko B. and Alexanian R. Cellular contents as a marker of human multiple myeloma. Blood 55, 403-8, 1980.

Lee EY-HP., Hu N., Yuan S.S., Cox L.A., Bradley A., Lee W.H. and Herrup K. Dual roles of the retinoblastoma protein in cell cycle regulation and neuron differentiation. Genes Dev 8, 2008-21, 1994.

Leech S.H., Brown R. and Schanfield M.S. Genetic studies in multiple myeloma. Immunoglobulin allotype associations. Cancer 55, 1473-6, 1985.

Leech S.H., Bryan C.F., Elston R.C., Rainey J., Bickers J.N. and Pelias M.Z. Genetic studies in multiple myeloma. Association with HLA Cw5. Cancer 51, 1408-11, 1983.

Lemoli R.M., Fortuna A., Grande A., Gamberi B., Bonsi L., Fogli M. et al. Expression and functional role of c-kit ligand (SCF) in human multiple myeloma cells. Br J Haematol 88, 760-9, 1994.

Lenhard R.E., Enterline J.P., Crowley J. and Ho G.Y.F. The effects of distance from primary treatment centres on survival among patients with multiple myeloma. J Clin Oncol 5, 1640, 1987.

Leo R., Boeker M., Peest D., Hein R., Bartl R., Gessner J.E. et al. Multiparameter analysis of normal and malignant human plasma cells: CD38++, CD56+, CD54+, cIg+ is the common phenotype of myeloma cells. Ann Hematol 64, 132-9, 1992.

Levi F. and La Vecchia C. Trends in multiple myeloma (letter). Int J Cancer 46, 755-6, 1990.

Levine A.M. Acquired immunodeficiency syndrome-related lymphoma. Blood 80, 8-20, 1992.

Levine A.M. and Gill P.S. AIDS-related malignant lymphoma: clinical presentation and treatment approaches. Oncology 1, 41-6, 1987.

Lewis D.R., Pottern L.M., Brown L.M., Silverman D.T., Hayes R.B., Schoenberg J.B. et al. Multiple myeloma among Blacks and Whites in the United States : the role of chronic antigenic stimulation. Cancer Causes and Control 5, 529-39, 1994.

Lewis E.B. Leukaemia, multiple myeloma and aplastic anemia in American radiologists. Science 142, 1492, 1963.

Lewis J.P. and MacKenzie M.R. Non-random chromosomal aberrations associated with multiple myeloma. Hematol Oncol 2, 307-17, 1984.

Ligthart G.J., Radl J., Corberand J.X., Van Nieuwkoop J.A., Van Staalduinen G.J. et al. Monoclonal gammopathies in human aging: Increased occurrence with age and correlation with health status. Mech Ageing Dev 52, 235, 1990.

Linet M.S., McLaughlin J.K., Hsing A.W., Wacholder S., Co-Chien H.T., Schuman L.M. et al. Is cigarette smoking a risk factor for non-Hodgkin's lymphoma or multiple myeloma? Results from the Lutheran Brotherhood Cohort Study. Leukemia Res 16, 621-4, 1992.

Linet M.S., McLaughlin J.K., Harlow S.A. and Fraumeni J.F. Family history of autoimmune disorders and cancer in multiple myeloma. Int J Epidemiol 17, 512-3, 1988.

Linet M.S., Harlow S.D. and McLaughlin J.K. A case-control study of multiple myeloma in whites: Chronic antigenic stimulation, occupation and drug use. Cancer Res 47, 2978, 1987.

Linos A., Kyle R.A., O'Fallon W.M. and Kurland L.T. Incidence and secular trend of multiple myeloma in Olmsted County, Minnesota: 1965-77. J Natl Cancer Inst 66, 17-20, 1981.

Linsley P.S. and Ledbetter P.S. The role of the CD28 receptor during T cell responses to antigen. Annual Review of Immunology 11, 191-212, 1993.

Lipinski M., Hirsch M.R., Deagostini-Bazin H., Yamada O., Tursz T. and Goridis C. Characterization of neural cell adhesion molecules (N-CAM) expressed by Ewing and neuroblastoma cell lines. Int J Cancer 40, 81-6, 1987.

Liu P., Leong T., Quam L., Billadeau D., Kay N.E., Greipp P. et al. Activating mutations of N- and K- ras in multiple myeloma show different clinical associations: Analysis of the Eastern Cooperative Oncology Group phase III trial. Blood 88, 2699, 1996.

Liu Y., Szekely L., Grander D., Soderhall S.I., Juliusson G., Gahrton G. et al. Chronic lymphocytic leukemia cells with allelic deletions at 13q14 commonly have one intact Rb1 gene: Evidence for a role of an adjacent locus. Proc Natl Acad Sci USA 90, 8697, 1993.

Loth T.S., Perrotta A.L., Lima J., Whiteaker R.S. and Robinson A. Genetic aspects of familial multiple myeloma. Mil Med 156, 430-3, 1991.

Ludwig H., Fritz E. and Friedl H.P. Epidemiologic and age-dependent data on multiple myeloma in Austria. J Natl Cancer Inst 68, 729-33, 1982.

Ludwig H. and Mayr W.R. Genetic aspects of suspectibility to multiple myeloma. Blood 59, 1286, 1982.

Lundberg I. Mortality and cancer incidence among Swedish paint industry workers with long-term exposure to organic solvents. Scand J Work Environ Health 12, 108-13, 1986.

MacKenzie J., Sheldon J., Morgan G., Cook G., Schulz T.F. and Jarret R.F. HHV-8 and multiple myeloma in the UK. Lancet 350, 1144-5, 1997.

MacLennan I.C.M., Hardie D.L., Ball J. and Drew M. Antibody-secreting cells and their origins. In Myeloma: Biology and Management. Eds. J.S. Malpas, D.E. Bergsagel and R.A. Kyle. Oxford University Press Inc., New York, 1995, p.30-62.

MacMahon B. Epidemiology of Hodgkin's disease. Cancer Res 26, 1189-200, 1966.

MacMahon B. and Clark D.W. The incidence of multiple myeloma. J Chron Dis 4, 508, 1956.

Magnus-Levy A. Multiple myeloma. Acta Med Scand 95, 217-18, 1938.

Malacrida V., De Francesco D., Banfi G., Porta F.A. and Riches P.G. Laboratory investigation of monoclonal gammopathy during 10 years of screening in a general hospital. J Clin Path 40, 793-7, 1987.

Malpas J.S. Clinical presentation and diagnosis. In Myeloma: Biology and Management. Eds. J.S. Malpas, D.E. Bergsagel and R.A. Kyle. Oxford University Press, Inc., New York, 1995, p.169-190.

Mancuso T.E., Stewart A. and Kreale G. Radiation exposure of Hanford workers dying from cancer and other causes. Health Phys 33, 369, 1977.

Mandema E. and Wildervanck L.S. La Maladie de Kahler. J Génét Hum 3, 170-5, 1954.

Mansa B. and Kjems E. Extremely high content of anti-α-staphylolysin in serum samples from a patient with multiple myeloma. Acta Patho Microbiol Scand 65, 303-10, 1965.

Manthorne L.A., Dudley R.W., Case C.D., Turgeon W.P. and Ritchie R.F. A longitudinal study of monoclonal gammopathy of undetermined significance (MGUS). Clin Res 36, 414A, 1988.

Marcelin A.G., Dupin N., Bouscary D., Bossi P., Cacoub P., Ravaud P. et al. HHV-8 and multiple myeloma in France. Lancet 350, 1144, 1997.

Marsh G.M., Enterline P.E. and McCraw D. Mortality patterns among petroleum refinery and chemical plant workers. Am J Ind Med 19, 29, 1991.

Mason D.Y. and Cullen P. HLA Antigen Frequencies in Myeloma. Tissue Antigens 5, 238-45, 1975.

Masood R., Zheng T., Tulpule A., Arora N., Chatlynne L., Handy M. et al. Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. Science 278, 1970-1, 1997.

Matanoski G.M. Risk of cancer associated with occupational exposure in radiologists and other radiation workers. In Cancer, Achievements, Challanges and Prospects for the 1980's. Eds. J.H. Burchenal and H.F. Ottegen. Grune and Stratton, New York 1982, p.24.

Mattiuz P.L., Ihde D., Piazza T., Ceppellini R. and Bodmer W.F. New approaches to the population genetic and serogenetic analysis of the HLA system. In Histocompatibility Testing. Ed. P.I. Terasaki. 1970, p.193-205.

McCrea A.P. and Morris T.C.M. Concurrent Familial Myeloma in Northern Ireland. Cancer 58, 394-6, 1986.

McLaughlin J.K., Linet M.S., Stone B.J., Blot W.J., Fraumeni J.F.Jr., Malker H.S.R. et al. Multiple myeloma and occupation in Sweden. Arch Environ Health 43, 7-10, 1988.

McPhedran P., Heath C.W.Jr. and Garcia J. Multiple myeloma incidence in metropolitan Atlanta, Georgia: Racial and seasonal variations. Blood 39, 866-73, 1972.

McWhorter W.P., Schatzkin A.G., Horm J.W. and Brown C.C. Contribution of socio-economic status to Black /white differences in cancer incidence. Cancer 63, 982, 1989.

Mellors R.C. Autoimmune disease in NZB/B1 mice. J Exp Med 129, 1045, 1969.

Merlini G. and Aguzzi F. The laboratory investigation of monoclonal components. Haematol 73, 79-85, 1988.

Metcalf D. The serum factor stimulating colony formation in vitro by murine plasmacytoma cells: Response to antigens and mineral oil. J Immunol 113, 235-43, 1974.

Meyerding H.W. Multiple Myeloma. Radiology 5, 132-6, 1925.

Milham S. Leukemia and multiple myeloma in farmers. Am J Epidemiol 94, 307-10, 1971.

Miller G., Rigsby M.O., Heston L., Grogan E., Sun R., Metroka C. et al. Antibodies to butyrate-induced antigens of Kaposi's sarcoma-associated herpesvirus in patients with HIV-1 infection. N Engl J Med 334, 1292-7, 1996.

Miller W.V. HL-A antigens and hematologic malignancy. Arch Intern Med 133, 397-9, 1974.

Mills P.K., Newell G.R., Beeson W.L., Fraser G.E. and Phillips R.L. History of cigarette smoking and risk of leukemia and myeloma : Results from the Adventist Health Study. J Natl Cancer Inst 82, 1832-6, 1990.

Minn A.J., Boise L.H. and Thompson C.B. Bcl-x(S) antagonizes the protective effects of Bcl-x(L). J Biol Chem 271, 6306-12, 1996.

Mitelman F. Catalog of Chromosome Aberrations in Cancer, 4th ed. New York, NY, Liss, 1991.

Mitterer M., Mair W., Gatti D., Sheldon J., Vachula M., Cosed P. et al. Dendritic cells derived from bone marrow and $CD34^+$ selected blood progenitor cells of myeloma patients, cultured in serum-free media, do not contain the Kaposi's sarcoma herpesvirus genome. Br J Haematol 102, 1338-40, 1998.

Monson R.R. and Nakano K.K. Mortality among rubber workers. II. Other employees. Am J Epidemiol 103, 297, 1976.

Moore P.S. Human herpesvirus 8 variants. Lancet 350, 679, 1998.

Moore P.S., Boshoff C., Weiss R.A. and Chang Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science 274, 1739-44, 1996.

Moore P.S. and Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infection. N Engl J Med 332, 1181-5, 1995.

Morgan R.J., Gonchoroff N.J., Katzmann J.A., Witzig T.E., Kyle R.A. and Greipp P.R. Detection of hypodiploidy using multiparameter flow cytometric analysis: a prognostic indicator in multiple myeloma. Am J Hematol 30, 195-200, 1989.

Morris P.D., Koepseli T.D., Daling J.R., Taylor J.W., Lyon J.L., Swanson G.M. et al. Toxic substance exposure and multiple myeloma : A case-control study. J Natl Cancer Inst 76, 987, 1986.

Muir C., Waterhouse J., Mack T., Powell J. and Whalan S. Cancer incidence in five continents, Volume V. IARC Scientific Publication No 88, International Agency for Research on Cancer, Lyon, France, 1987.

Mukiibi J.M. and Kyobe J. Pattern of multiple myeloma in Kenyans. Trop Geogr Med 40, 20, 1988.

Mukiibi J.M. and Mkwananzi J.B. Multiple myeloma in Zimbabweans I. E Afr Med J 64, 471-81, 1987.

Mukiibi J.M., Karimi M. and Kyobe J. Multiple myeloma in Kenyan Africans - A prospective study. E Afr Med J 58, 171-9, 1981.

Muylle L., Desmet D., Cole J. and Peetermans M.E. HLA-DR and monoclonal gammopathy. Tissue Antigens 20, 397-9, 1982.

Nandakumar A., English D.R., Dougan L.E. and Armstrong B.K. Incidence and outcome of multiple myeloma in Western Australia, 1960 to 1984. Aust NZ J Med 18, 774-9, 1988.

Nandakumar A., Armstrong B.K. and de Klerk N.H. Multiple myeloma in Western Australia. A case-control study in relation to occupation, father's occupation, socio-economic status and country of birth. Int J Cancer 37, 223, 1986.

Neipel F., Albrecht J.C. and Fleckenstein B. Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenecity? J Virol 71, 4187-92, 1997.

Neri A., Baldini L., Trecca D., Cro L., Polli E. and Maiolo A.T. p53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. Blood 81, 128, 1993.

Neri A., Murphy J.P., Cro L., Ferrero D., Tarella C., Baldini L. et al. Ras oncogene mutations in multiple myeloma. J Exp Med 170, 1715, 1989.

Neriishi K., Yoshimoto Y., Carter R.L., Matsou T., Ichimaru M., Abe T. et al Monoclonal gammopathy in atomic bomb survivors. Radiat Res 133, 351-9, 1993.

Nobuyoshi M., Kawano H., Tanaka H., Ishikawa H., Tanabe O., Iwato K. et al. Increased expression of the c-myc gene may be related to the aggressive transformation of human myeloma cells. Br J Haematol 77, 523, 1991.

Nogués X., Supervia A., Knobel H. and Lopez-Colomés J.L. Multiple myeloma and AIDS. Am J Hematol 53(3), 210-11, 1996.

Norusis M.J. Kaplan-Meier Survival Analysis. In Advanced Statistics 6.1 Ed. M.J. Norusis. Chicago, Illinois, 1994, p.275-86.

Nosari A.M., Landonio G., Cantoni S., Errante D., Volonterio A. and Morra E. Multiple myeloma associated to HIV infection : report of two patients. Eur J Haematol 56, 98-9, 1996.

Nunez G., London L., Hockenberg D., Alexander M., McKearn J.P. and Korsmeyer S.J. Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. J Immunol 144, 3602-10, 1990.

Odeberg H., Johannson B.G. and Berlin S-O. Immunoglobulin analysis in families of myeloma patients. Acta Med Scand 196, 361-7, 1974.

Olsen S.J., Tarte K., Sherman W., Hale E, Weisse M., Orazi A. et al. Evidence against KSHV infection in the pathogenesis of human myeloma. Virus Res 57, 197-202, 1998.

Olshan A.F. Familial and Genetic Associations. In Epidemiology and Biology of Multiple Myeloma. Eds. G.I. Obrams and M. Potter. Springer-Verlag, Berlin, 1991, p.31-9.

Oltvai Z.N., Milliman C.L. and Korsmeyer S.J. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74, 609-19, 1993.

Omedè P., Boccadoro M., Fusaro A., Gallone G. and Pileri A. Multiple myeloma: 'early' plasma cell phenotype identifies patients with aggressive biological and clinical characteristics. Br J Haematol 85, 504-13, 1993.

Omedè P. and Pileri A. C-reactive protein (CRP): a direct indicator of interleukin-6 and the best prognostic factor in multiple myeloma (MM). Blood 78 (suppl 1), 174a, 1991.

Onyemelukwe G.C. and Kulkarni A.G. Immunoglobulin types and features of Multiple Myeloma in Northern Nigeria. E Afr Med J 65, 33-8, 1988.

Orenstein J.M., Fox C. and Wahl S.M. Macrophages as a source of HIV during opportunistic infections. Science 276, 1857-61, 1997.

Orfão A., García-Sanz R., López-Berges M.C., Vidriales M.B., Gonzalez M. Caballero M.D. and San Miguel J.F. A new method for the analysis of plasma cell DNA content using a CD38/propidium iodide double staining technique. Cytometry 17, 332-9, 1994.

Ott M.G., Teta M.J. and Greenberg H.L. Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. Am J Ind Med 16, 631, 1989.

Pahwa S., Pahwa R., Good R.A., Gallo R.C. and Saxinger C. Stimulatory and inhibitory influences of human immunodeficiency virus on normal B lymphocytes. Proc Natl Acad Sci USA 83, 9124-8, 1986.

Parravicini C., Lauri E., Baldini L., Neri A., Poli F., Sirchia G. et al. Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. Science 278, 1969, 1997.

Parker S.L., Tong T., Bolden S. and Wingo P.A. Cancer Statistics. CA Cancer J Clin 47(1), 5-27, 1997.

Parkin D.M., Muir C.S., Whelan S.L., Gao Y.T., Ferlay J. and Powell J. Cancer Incidence in Five Continents, Volume VI. International Agency for Research on Cancer. Scientific Publication No 120. Lyon, France, 1992.

Parkin D.M. Cancer Occurrence in Developing Countries. IARC Scientific Publication No 75. Lyon, France, 1986.

Pasqualetti P., Colantonio D., Collaciani A. and Casale R. Stato socioeconomico e sopravvivenza nel mieloma multiplo. Minerva Med 81, 713, 1990.

Passweg J., Thiel G. and Bock H.A. Monoclonal gammopathy after intense induction immunosuppression in renal transplant patients. Nephrol Dial Transplant 11(12), 2461-5, 1996.

Patel M. Haematology. In Baragwanath Hospital - A Medical Miscellany. Eds. K. Huddle and A. Dubb. Ultra Litho, 173-90, 1994.

Patel M., Celliers L., Dukes I.A.F., Fleming A.F., Stevens W., Harku V. et al. Multiple Myeloma at Baragwanath Hospital. Abstract No. 875. International Society of Haematology Congress, London, 1992, p.230.

Paxton M.B., Chinchilli V.M., Brett S.M. and Rodricks J.V. Leukemia risk associated with benzene exposure in the pliofilm cohort: I. Mortality update and exposure distribution . Risk Anal 14, 147-54, 1994.

Payne G.S., Bishop J.M. and Varmus H.E. Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. Nature 295, 209, 1982.

Pearce N., Prior I., Metuvan D., Culling C., Marshall S., Auld J. et al. Follow up of New Zealand participants in British atmospheric nuclear weapons tests in the Pacific. BMJ 300, 1161-6, 1990.

Pearce N. and Reif J.S. Epidemiologic studies of cancer in agricultural workers. Am J Ind Med 18, 133-48, 1990.

Pearce N.E., Smith A.H., Howard J.K., Sheppard R.A., Giles H.J. and Teague C.A. Case-control study of multiple myeloma and farming. Br J Cancer 54, 493, 1986.

Pearce N.E., Smith A.H. and Fisher D.O. Malignant lymphoma and multiple myeloma linked with agricultural occupations in a New Zealand cancer registry-based study. Am J Epidemiol 121, 225-37,1985.

Pellat-Deceunynck C., Barille S., Puthier D., Rapp M-J., Harousseau J-L., Bataille R. and Amiot M. Adhesion molecules on human myeloma cells: significant changes in expression related to malignancy, tumour spreading and immortalization. Cancer Res 55, 3647-53, 1995.

Pellat-Deceunynck C., Bataille R., Robillard N., Harousseau J-L., Juge-Morineau N. Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells. Blood 84, 2597-603, 1994.

Pérez-Simón J.A., García-Sanz R., Tabernero M.D., Almeida J., González M., Fernández-Calvo, et al. Prognostic value of numerical chromosome aberrations in multiple myeloma: A FISH analysis of 15 different chromosomes. Blood 91, 3366-71, 1998.

Pick A.I., Schoenfeld Y., Skvuril F., Schreibman S., Frohlielunan R., Weiss H. et al. Asymptomatic (benign) monoclonal gammopathy - a study of 100 patients. Ann Clin Lab Sci 7, 335-43, 1977.

Pietruszka M , Rabin B.S. and Srodes C. Multiple myeloma in husband and wife. Lancet 1, 314, 1976.

Pilarski L.M. and Jensen G.S. Monoclonal circulating B cells in multiple myeloma: a continuously differentiating, possibly invasive, population as defined by expression of CD45 isoforms and adhesion molecules. In Multiple Myeloma. Hematol Oncol Clin North Am. Ed. B. Barlogie. Philadelphia, PA: WB Saunders, 1992, p.297-322.

Pileri A. and Tarocco R.P. In vivo kinetic studies in human myeloma. Haematologica 59, 10-24, 1974.

Pilgrim H.I. The relationship of chronic ulceration of the ileocecal junction to the development of reticuloendothelial tumours in C_3H mice. Cancer Res 25, 53, 1965.

Piras M.A., Salis T., Naitana A.V., Spanu L. and Aceti A. HIV infection and neoplasia. Lancet 348, 1316-7, 1996.

Porter D.D., Dixon F.J. and Larsen A.E. The development of a myeloma-like condition in mink with Aleutian disease. Blood 25, 736-42, 1965.

Potter M. Concepts of pathogenesis and experimental models of Immunoglobulin secreting tumors in animals. In Neoplastic Diseases of the Blood. Vol 1. Eds. P.H. Wiernik, G.P. Canellos, R.A. Kyle and C.A. Schiffer. (1st ed.). Churchill Livingstone, New York 1985, p.393-412.

Potter M. Antigen-binding myeloma proteins of mice. Adv Immunol 25, 141, 1977.

Potter M., Pumphrey J.G. and Bailey D.W. Genetics of susceptibility of plasmacytoma induction. I. BALB/cAnN (C), C57BL/6N (B6), C57BL/Ka (BK), (CxB6) F1, (CxBK) F1 and CxB recombinant-inbred strains. J Natl Cancer Inst 54, 1413-17, 1975.

Potter M. The developmental history of the neoplastic plasma cell in mice. A brief review of recent developments. Semin Hematol 10, 19, 1973.

Potter M. Immunoglobulin producing tumors and myeloma proteins in mice. Physiol Rev 52, 631, 1972.

Potter M. Myeloma proteins (M-components) with antibody-like activity. N Engl J Med 284, 831-8, 1971.

Potter M. and Leon M.A. Three IgA myeloma immunoglobulins from the BALB/c mouse: precipitation with pneucomococcal C polysaccharide. Science 162, 369, 1968.

Potter M. Plasma cell neoplasia in a single host: A mosaic of different protein-producing cell types. J Exp Med 115, 339, 1962.

Potter M. and Boice C.R. Induction of plasma-cell neoplasms in strain BALB/c mice with mineral oil and mineral oil adjuvants. Nature 193, 1086, 1962.

Potter M. and Robertson C.L. Development of plasma-cell neoplasms in BALB/c after intraperitoneal injection of paraffin oil adjuvant, heat killed staphylococcus mixtures. J Natl Cancer Inst 25, 847, 1960.

Pottern L.M., Heineman E.F., Olsen J.H., Raffn E. and Blair A. Multiple myeloma among Danish women: empolyed history and work place exposures. Cancer Causes and Control 3, 427-32, 1992a.

Pottern L.M., Gart J.J., Nam J-M., Dunston G., Wilson J., Greenberg R. et al. HLA and multiple myeloma among Black and White men: evidence of a genetic association. Cancer Epidemiol Biomarkers Prev 1, 177-82, 1992b.

Preston D.L., Kusumi S., Tomonaga M., Izumi S., Ron E., Kuramoto A. et al. Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 137, S68-97, 1994.

Pukkala E. and Notkola V. Cancer incidence among Finnish farmers, 1979-1993. Cancer Causes and Control 8(1), 25-33, 1997.

Rabkin C.S., Schulz T.F., Whitby D., Lennette E.T., Magpantay L.I., Chatlynne L. et al. Interassay correlation of HHV-8 serologic tests. J Infect Dis 178, 304-9, 1998.

Radl J. and Hollander C.F. Homogenous immunoglobulins in the sera of mice during aging. J Immunol 112, 2271-3, 1974.

Raffeld M. and Jaffe E.S. BCL-1, t(11;14) and mantle cell-derived lymphomas. Blood 78, 259, 1991.

Raffn E., Lynge E., Juel K. and Korsgaard B. Incidence of cancer and mortality among employees in the asbestos cement industry in Denmark. Br J Ind Med 46, 90-6, 1989.

Rawstron A., Barrans S., Blythe D., Davies F., English A., Pratt G. et al. Distribution of myeloma plasma cells in peripheral blood and bone marrow correlates with CD56 expression. Br J Haematol 104, 138-43, 1999.

Rees R.C. MHC restricted and non-restricted killer lymphocytes. Blood Reviews 4, 204-10, 1990.

Reichardt V., Okada C., Benike C., Long G., Engleman E., Blume K. et al. Idiotype vaccination using dendritic cells for multiple myeloma patients after autologous peripheral stem cell transplantation. Blood 88, 481a, 1996.

Reif J.S., Pearce N.E. and Fraser J. Cancer risks among New Zealand meat workers. Scand J Work Environ Health 15, 24, 1989.

Renier G., Renier J.C., Gardembas-Pain A., Chavailler A., Boasson M. and Hurez D. Ankylosing spondylitis and monoclonal gammopathies. Ann Rheum Dis 51, 951-4, 1992.

Rettig M.B., Vescio R.A., Ma H.J., Moss T., Schiller G., Said J. et al. Detection of Kaposi's sarcoma-associated herpesvirus in the dendritic cells of Waldenstrom's macroglobulinemia and primary amyloidosis patients. Blood 90, 86a, 1997a(suppl 1).

Rettig M.B., Ma H.J., Vescio R.A., Pold M., Schiller G., Belson D. et al. Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. Science 276, 1851-4, 1997b.

Richards S.J. and Scott C.S. Human NK cell in health and disease: clinical, functional, phenotypic and DNA genotypic characteristics. Leuk Lymphoma 7, 377-99, 1992.

Riedel D.A. and Pottern L.M. The Epidemiology of Multiple Myeloma. In Haematol Oncol Clin N Am. Multiple Myeloma. Ed. B. Barlogie. 6, 1992, p.225-47.

Riedl D.A., Pottern L.M. and Blattner W.A. Epidemiology of multiple myeloma. In Neoplastic Diseases of the Blood. Eds. P.H.Wiernik, G.P.Canellos, R.A. Kyle and C.A.Schiffer. (2nd ed.). Churchill Livingstone, New York, 1991, p.347-72.

Ries L.A.G., Miller B.A., Hankey B.F., Kosary B.L., Harras A. and Edwards B.K. SEER Cancer Statistics Review, 1973-1991: Tables and Graphs, National Cancer Institute Publication No 94-2789. US Government Printing Office, Washington, DC, 1994.

Ries L.A.G., Hankey B.F., Miller B.A., Hartman A.M. and Edwards B.K. Cancer Statistics Review 1973-1988 (DHHS Publ (NIH) No 91-2789). National Cancer Institute, Bethesda, Maryland, 1991.

Rinsky R.A., Smith A.B., Hornung R., Fillon T.G., Young R.J., Okun A.H. et al. Benzene and leukemia : an epidemiological risk assessment. N Engl J Med 316, 1044-50, 1987.

Robertson M J. and Ritz J. Biology and clinical relevance of human natural killer cells. Blood 76, 2421-38, 1990.

Roth J., Zuber C., Wagner P., Taatjes D.J., Weisgerber C., Heitz P.U. et al. Reexpression of poly (sialic acid) units of the neural cell adhesion molecule in Wilms tumor. Proc Natl Acad Sci USA 85, 2999-3003, 1988.

Ruiz-Argüelles G.J. and San Miguel J.F. Cell surface markers in multiple myeloma. Mayo Clinic Proceedings 69, 684-90, 1994.

Russo J.J., Bohenzky R.A., Chien M.C., Chen J., Yan M., Maddalena D. et al. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proc Natl Acad Sci USA 93, 14862-7, 1996.

Ryan K.M. and Birnie G.D. Myc oncogenes: The enigmatic family. Biochem J 314, 713, 1996.

Said J.W., Rettig M.R., Heppner K., Vescio R.A., Schiller G., Ma H.J. et al. Localization of Kaposi's sarcoma-associated herpesvirus in bone marrow biopsy samples from patients with multiple myeloma. Blood 90, 4278-82, 1997.

Sala P., Mazzolini S., Tonutti E. and Bramezza M. Monoclonal immunoglobulins in HTLV-III positive sera. Clin Chem 32, 574, 1986.

Saleün J.P., Vicariot M. and Deroff P. Monoclonal gammopathies in the adult population of Finistère, France. J Clin Pathol 35, 63, 1982.

Saleün J.P., Youinou P., Le Goff P., Le Menn G. and Morin J.F. HLA Antigens and Monoclonal Gammopathy. Tissue Antigens 13, 233-5, 1979.

Salmon S.E. and Seligmann M. B-cell neoplasia in man. Lancet 2, 1230, 1974.

San Miguel J.F., Garcia-Sanz R., Gonzalez M. and Orfao A. Immunophenotype and DNA cell content in multiple myeloma. In Bailliere's Clinical Haematology. Multiple Myeloma. Ed. F. Mandelli. Bailliere Tindall. 8 (4), 1995, p.735-59.

San Miguel J.F., Gonzalez M., Gascon A., Moro M.J., Hernandez J.M., Ortega F. et al. Immunophenotype heterogeneity of multiple myeloma: influence on the biology and clinical course of the disease. Br J Haematol 77, 185-90, 1991.

San Miguel J.F., Caballero M.D., Gonzalez M., Zola H. and Lopez-Borrasca A. Immunological phenotype of neoplasms involving the B cell in the last step of differentiation. Br J Haematol 62, 75-83, 1986.

Sandberg A.A. The chromosomes in Human Cancer and Leukaemia. (2nd ed.). Elsevier Science, New York, 1990.

Santhanam U., Ray A. and Sehgal P.B. Repression of the interleukin 6 gene promoter by p53 and the retinoblastoma susceptibility gene product. Proc Natl Acad Sci USA 88, 7605, 1991.

Savage D., Lindenbaum J., Van Ryzin J., Struening E. and Garrett T.J. Race, poverty and survival in multiple myeloma. Clin Res 31, 365A, 1983.

Savitz D.A. and Andrews K.W. Review of epidemiologic evidence on benzene and lymphatic and hematopoietic cancers. Am J Ind Med 3 (3), 287-95, 1997.

Sawyer J.R., Lukacs J.L., Munshi N., Desikan K.R., Singhal S., Mehta J. et al. Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. Blood 92, 4269, 1998.

Sawyer J.R., Waldron J.A., Jagannath S. and Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet 82, 41-9, 1995.

Sawyer J.R., Tricot G., Mattox S., Jagannath S., Vesole D. and Barlogie B. Monosomy 13 (-13) or abnormalities involving 14q are associated with poor prognosis in multiple myeloma (MM). Blood 84, 523a, 1994 (Suppl 1).

Schafer A.I. and Miller J.B. Association of IgA multiple myeloma with pre-existing disease. Br J Haematol 41, 19-24, 1979.

Schechter G.P., Shoff N., Chan C. and Hawley H.P. The frequency of monoclonal gammopathy of undetermined significance (MGUS) in Black and Caucasian veterans in a hospital population (abstract). Blood 76 (1), 371a, 1990

Schnittman S.M., Lane H.C., Higgins S.E., Folks T. and Fauci A.S. Direct polyclonal activation of human B-lymphocytes by the acquired immunodeficiency syndrome virus. Science 233, 1084-6, 1986.

Schulz T.F., Boshoff C.H. and Weiss R.A. HIV infection and neoplasia. Lancet 348, 587-91, 1996.

Schwartz E. A proportionate mortality ratio analysis of pulp and paper mills workers in New Hampshire. Br J Med 45, 234, 1988.

Schwartz G.G. Multiple myeloma: clusters, clues and dioxins. Cancer Epidemiol Biomarkers Prev 6(1), 49-56, 1997.

Schwartz R.H. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell 71, 1065, 1992.

Selby J.V., Friedman G.D. and Fireman B.H. Screening prescription drugs for possible carcinogenicity: Eleven to fifteen years of follow up. Cancer Res 49, 5736, 1989.

Seligmann M., Danon F., Basch A. and Bernard J. IgA myeloma cryoglobulin with antistreptolysin activity. Nature 220, 711-2, 1968.

Selvanayagam P., Blick M., Narni F., van Tuinen P., Ledbetter D.H., Alexanian R. et al. Alterations and abnormal expression of the c-myc oncogene in human multiple myeloma. Blood 71, 30-5, 1988.

Seong C., Delasalle K., Hayes K., Weber D., Dimopoulos M., Swantkowski J. et al. Prognostic value of cytogenetics in multiple myeloma. Br J Haematol 101, 189-94, 1998.

Shapira R. and Carter A. Multiple myeloma in northern Israel, 1970-1979. Cancer 58, 206-9, 1986.

Shapiro G.I., Edwards C.D., Kobzik L., Goldleski J., Richards W., Sugarbaker D.J. et al. Reciprocal Rb inactivation and $p16^{INK4}$ expression in primary lung cancer cell lines. Cancer Res 55, 505, 1995.

Shimazaki C., Atzpodien J, Wisniewsky D., Gulati S.C., Kolitz J.E., Fried J. and Clarkson B.D. Cell-mediated toxicity of interleukin-2 activated lymphocytes against autologous and allogeneic human myeloma cells. Acta Haematol 80, 203-9, 1988.

Shimizu Y., Kato H. and Schull W.J. Studies of the mortality of A-bomb survivors. 9. Mortality, 1950-1985: Part II. Cancer mortality based on the recently revised doses (DS86). Radiat Res 121, 120-41, 1990.

Shulman G. and Jacobson R.J. Immunocytoma in black and white South Africans. Trop Geogr Med 32, 112, 1980.

Shulman G., Gilich G.C. and Andrew M.J.A. Serum Immunoglobulins G, A and M in White and Black Adults on the Witwatersrand. S Afr Med J 49, 1160-4, 1975.

Simonsson B., Kallander C.F., Brenning G., Killander A., Gronowitz J.S. and Bergstrom R. Biochemical markers in multiple myeloma: a multivariate analysis. Br J Haematol 69, 47-53, 1988.

Simpson G.R., Schulz T.F., Whitby D., Cook P.M., Boshoff C., Rainbow L. et al. Prevalence of Kaposi's sarcoma-associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 348, 1133-8, 1996.

Singh J., Dudley A.W.Jr. and Kulig K.A. Increased incidence of monoclonal gammopathy of undetermined significance in blacks and its age related differences with whites on the basis of a study of 397 men and women in a hospital setting. J Lab Clin Med 116, 785, 1990.

Sitas F., Carrara H., Beral V., Newton R., Reeves G., Bull D. et al. The seroepidemiology of HHV-8/KSHV in a large population of black cancer patients in South Africa. N Engl J Med 340, 1863-71, 1999.

Sitas F., Terblanche M. and Madhoo J. Incidence and Geographical Distribution of Histologically Diagnosed Cancer in South Africa, 1990 & 1991. National Cancer Registry Publication, 1996.

Slack R.S., Hamel P.A., Bladon T.S., Gill R.M. and McBurney M.W. Regulated expression of the retinoblastoma gene in differentiating embryonal carcinoma cells. Oncogene 8, 1585, 1993.

Smith G., Walford R.L., Fishkin B., Carter P.K. and Tanaka K. HLA phenotypes, immunoglobulins and κ and λ light chains in multiple myeloma. Tissue Antigens 4, 374-7, 1974.

Smith P.G. and Douglas A.J. Mortality of workers at the Sellafield plant of British Nuclear Fuels. BMJ 293, 845, 1986.

Snyder R., Wix G. and Goldstein B.D. The toxicology of benzene. Environ Health Persp 100, 295, 1993.

Sorahan T., Parkes H.G., Veys C.A., Waterhouse J.A.H., Straughhan J.K. and Nutt A. Mortality in the British rubber industry 1946-85. Br J Ind Med 46, 1-10, 1989.

Soulier J., Grollet L., Oksenhendler E., Cacoub P., Cazals-Hatem D., Babinet P. et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 86, 1276-80, 1995.

Spinelli J.J., Gallagher R.P., Band P.R. and Threlfall W.J. Multiple myeloma, leukemia, and cancer of the ovary in cosmetologists and hairdressers. Am J Ind Med 6, 97-102, 1984.

Spirtas R., Stewart P.A., Lee J.S., Marano D.E., Forbes C.D., Grauman D.J. et al. Retrospective cohort mortality study of workers at an aircraft maintenance facility. 1. Epidemiological results. Br J Ind Med 48, 515-30, 1991.

Stebbings J.H., Lucas H.F. and Stehrey A.F. Mortality from cancers of major sites in female radium dial workers. Am J Ind Med 5, 435, 1984.

Stilgenbauer S., Dohner H., Bulgay-Morshel M., Weitz S., Bentz M. and Lichter P. High frequency of monoallelic retinoblastoma gene deletion in B-cell chronic lymphoid leukaemia shown by interphase cytogenetics. Blood 81, 2118, 1993.

Su I.J., Hsu Y.S., Chang Y.C. and Wang I.W. Herpes-like DNA sequence in Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. Lancet 345, 722-3, 1995.

Sumegi J., Hedberg T., Bjorkholm M., Sodal T., Mellstedt H., Nilsson M.G. et al. Amplification of the c-myc oncogene in human plasma cell leukaemia. Int J Cancer 36, 367-71, 1985.

Svensson B.G., Englander V., Akesson B., Attewell R., Skerfving S. and Ericson A. Deaths and tumors among workers grinding stainless steel. Am J Ind Med 15, 51-9, 1989.

Tabernero D., San Miguel J.F., Garcia-Sanz R., Najera L., Garcia-Isidora M., Perez-Simon J.A. et al. Incidence of chromosome numerical changes in multiple myeloma: Fluorescence in situ hybridization analysis using 15 chromosome-specific probes. Am J Pathol 149, 153, 1996.

Tafuri A., Meyers J., Lee B.J. and Andreeff M. DNA and RNA flow cytometric study in multiple myeloma. Clinical correlations. Cancer 67, 449-54, 1991.

Talerman A. Clinico-pathological study of multiple myeloma in Jamaica. Br J Cancer 23, 285-93, 1969.

Tarte K., Olsen S.J., Rossi J.F., Legouffe E., Lu Z.Y., Jourdan M. et al. Kaposi's sarcoma-associated herpesvirus is not detected with immunosuppression in multiple myeloma. Blood 92, 2186-8, 1998.

Tazzari P.L., Gobbi M. and Dinota A. Normal and neoplastic plasma cell membrane phenotype: studies with new monoclonal antibodies. Clin Exp Immunol 70, 192-200, 1987.

Terasaki P.I., McClelland J., Park M.S. and McCurdy B. Microdroplet lymphocyte cytotoxity test. In Manuel of tissue typing techniques. Eds. J.G. Ray., D.B. Hare., P.D. Pedersen and Mullally D.I. NIH, Bethesda, Maryland, 1974, p67-74.

Terstappen L.W.M.M., Johansen W., Segers-Nolten I.M.J. and Loken M.R. Identification and characterization of normal human plasma cells in normal human bone marrow by high resolution flow cytometry. Blood 76, 1739-47, 1990.

Tienhaara A. and Pelliniemi T.T. Flow cytometric DNA analysis and clinical correlations in multiple myeloma. Am J Clin Path 97, 322-30, 1991.

Tisdale J.F., Stewart A.K., Dickstein B., Dube I.D., Cappe D., Dunbar C.E. et al. Human herpesvirus 8 in patients with multiple myeloma. Blood 90, 588a, 1997(suppl 1).

Tollerud D.J., Brown L.M., Blattner W.A. and Hoover R.N. The influence of race on T-cell subset distributions. In Epidemiology and biology of multiple myeloma. Eds. G.I. Obrams and M. Potter. Springer-Verlag, New York, 1991, p.45-9.

Tollerud D.J., Brinton L.A., Stone B.J., Tobacman J.K. and Blattner W.A. Mortality from multiple myeloma among North Carolina furniture workers. J Natl Cancer Inst 74, 799-801, 1985.

Tominaga N., Katahari S. and Ohnishi M. Analysis of surface antigen expression of human immunoglobulin-secreting cells: phenotype heterogeneity in normal counterparts of myeloma cells. Br J Haematol 73, 302-8, 1989.

Tong A.W., Zhang B.Q., Mues G., Solano M., Hanson T. and Stone M.J. Anti-CD40 antibody binding modulates human multiple myeloma clonogenecity in vitro. Blood 84, 3026-33, 1994.

Torres A., Giralt M. and Raichs A. Coexistencia de antecedentes benzólicos crónicosy plasmacitoma multiple. Presentación de dos casos. Sangre 15, 275, 1970.

Tozer R.A., Clear A.S., Davies D.R. and Hutt M.S.R. Unusual presentation of patients with myelomatosis in Malawi. J Clin Path 33, 544-6, 1980.

Tricot G., Barlogie B., Jagannath S., Bracy D., Mattox S., Vesole D.H. et al. Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. Blood 86, 4250-6, 1995.

Turbat-Herrera E., Hancock C., Cabello-Inchausti B. and Herrera G. Plasma cell hyperplasia and monoclonal paraproteinemia in human immunodeficiency virus infected patients. Arch Path Lab Med 117, 497-501, 1993.

Turesson I., Zettevall O., Cuzick J., Waldenstrom J.G. and Velez R. Comparison of trends in the incidence of multiple myeloma in Malmö, Sweden, and other countries, 1950-1979. N Engl J Med 310, 421-4, 1984.

Ucci G., Riccardi A., Luoni R. and Ascari E. Presenting features of monoclonal gammopathies: an analysis of 684 newly diagnosed cases. J Int Med 234, 165-73, 1993.

Ucci G., Petrini M. and Riccardi A. Expression of p170 protein in multiple myeloma: a clinical study. Hematol Oncol 10, 213-20, 1992.

Uckun F.M. Regulation of human B-cell ontogeny. Blood 76, 1908-23, 1990.

Vågerö D. and Persson G. Ocurrence of cancer in socioeconomic groups in Sweden: an analysis based on the Swedish Cancer Environment registry. Scand J Soc Med 14, 151-60, 1986.

Van Camp B., Durie B.G.M., Spier C., De Waele M., Van Riet I., Vella E. et al. Plasma cells in multiple myeloma express a natural killer-cell associated antigen: CD56 (NKH-1; Leu19). Blood 76, 377-82, 1990.

Van Camp B.G.K., Cole J. and Peetermans M.E. HLA antigens and homogenous immunoglobulins. Clin Immunol Immunopathol 7, 315-8, 1977.

Van Riet I., Bakkus M., De Greef C., Faid L. and Van Camp B. Homing mechanisms in the etiopathogenesis of multiple myeloma. Stem cells 13(suppl 2), 22-7, 1995.

Van Riet I. and Van Camp B. The involvement of adhesion molecules in the biology of multiple myeloma. Leuk Lymphoma 9, 441-52, 1993.

Van Riet I., De Waele M., Remels L., Lacor P., Schots R. and Van Camp B. Expression of cytoadhesion molecules (CD56, CD54, CD18, CD29) by myeloma cells. Br J Haematol 79, 421-7, 1991.

Velez R., Beral V. and Cuzick J. Increasing trends of multiple myeloma mortality in England and Wales: 1950-79: are changes real? J Natl Cancer Inst 69, 387-92, 1982.

Vescio R., Wu C., Rettig M.B., Stewart A., Ballester O., Noga S. et al. The detection of KSHV is increased by mobilisation chemotherapy and reduced in autografts by CD34-selection. Blood 90, 565a, 1997(suppl 1).

Vescio R.A., Hong C.H., Cao J., Kim A., Schiller G.J. and Lichtenstein A.K. The hematopoietic stem cell antigen, CD34, is not expressed on the malignant cells in multiple myeloma. Blood 84, 3283-90, 1994.

Vineas P. and The Working Group on the Epidemiology of Hematolymphopoietic Malignancies in Italy. Incidence and Time Trends for Lymphomas, Leukemias and Myelomas: Hypothesis Generation. Leukemia Res 20(4), 285-90, 1996.

Vladutiu A.O. Prevalence of M-proteins in serum of hospitalized patients. Physicians' response to finding M-proteins in serum protein electrophoresis. Ann Clin Lab Sci 17, 157, 1987.

Wadee A.A. and Dunn D.S. HLA-A, B and C polymorphisms in three major populations living in South Africa. Gene Geography 5, 23-32, 1991.

Walrath J., Decoulfe P. and Thomas T.L. Mortality among workers in a shoe manufacturing company. Am J Ind Med 12, 615, 1987.

Wang J-X., Boice J.D.Jr., Li B-X., Zhang J-Y. and Fraumeni J.F.Jr. Cancer among medical diagnostic X-ray workers in China. J Natl Cancer Inst 80, 344-50, 1988.

Warburton P., Joshua D.E., Gibson J. and Brown R.D. CD10-(CALLA)-positive lymphocytes in myeloma: evidence that they are a malignant precursor population and are of germinal centre origin. Leuk Lymphoma 1, 11-20, 1989.

Waterhouse J.A., Muir C., Shanmugaratnam K. and Powell J. Cancer Incidence in Five Continents, Volume IV. IARC Scientific Publication No 42, International Agency for Research on Cancer, Lyon, France, 1982.

Wegelius O. and Skrifvars B. Rheumatoid arthritis terminating in plasmacytoma. Acta Med Scand 187, 133-8, 1970.

Weh H.J., Gutensohn K., Selbach J., Kruse R., Wacker-Backhaus G., Seeger D. et al. Karyotype in multiple myeloma and plasma cell leukaemia. Eur J Cancer 29A, 1269, 1993.

Weinberg R.A. The retinoblastoma protein and cell cycle control. Cell 81, 323, 1995.

Weinberg R.A. The molecular basis of retinoblastomas. Ciba Foundation Symposia. 142, 99-105, 1989.

Weiss H.A., Darby S.C. and Doll R. Cancer mortality following X-ray treatment for ankylosing spondylitis. Int J Cancer 59(3), 327-38, 1994.

Weston B., Grufferman S., MacMillan J.P. and Cohen H.J. Effects of socioeconomic and clinical factors on survival of multiple myeloma. J Clin Oncol 5, 1977-84, 1987.

Whitby D., Luppi M., Barozzi P., Boshoff C., Weiss R.A. and Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 90, 395-7, 1998.

Whitby D., Howard M.R., Tonent-Flowers M., Brink N.S., Copas A., Boshoff C. et al. Detection of Kaposi's sarcoma-associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 346, 799-802, 1995.

Wiklund K., Dich J., Holm L-E. and Eklund G. Risk of cancer in pesticide applicators in Swedish agriculture. Br J Ind Med 46, 809, 1989.

Wiklund K. and Holm L-E. Trends in cancer risks among Swedish agricultural workers. J Natl Cancer Inst 77, 657, 1986.

Wijdenes J., Vooijs W.C., Clement C., Post J., Morard F., Vita N. et al. A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br J Haematol 94, 318-23, 1996.

Wilkinson D., Sheldon J., Gilks C.F. and Schulz T.F. Prevalence of infection with Human Herpesvirus-8/Kaposi's Sarcoma Herpesvirus in rural South Africa. S Afr Med J 89, 554-7, 1999.

Willems P.M., Kuypers A.W., Meijerink J.P., Holdrinet R.S. and Mensink E.J. Sporadic mutations of the p53 gene in multiple myeloma and no evidence for germline mutations in three familial multiple myeloma pedigrees. Leukaemia 7, 986-91, 1993.

Williams A.R., Weiss N.S., Koepsell T.D., Lyon J.L. and Swanson G.M. Infectious and non-infectious exposures in the etiology of light chain myeloma: a case-control study. Cancer Res 49, 4038-41, 1989.

Williams R.R. and Horm J.W. Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: interview study from the Third National Cancer Survey. J Natl Cancer Inst 58, 525-47, 1977.

Winchester R., Togushi T., Szer I., Burmester G., Lo Galbo P., Cuttner J.D. et al. Association of susceptibility to certain haematopoietic malignancies with the presence of Ia allodeterminants distinct from the DR series: Utility of monoclonal antibody reagents. Immunol Rev 70, 155-66, 1983.

Wolverkamp M.C.J. and Marquet R.L. Interleukin-6: historical background, genetics and biological significance. Immunology Letters 24, 1-10, 1990.

Wong O. and Raabe G.K. Multiple myeloma and benzene exposure in a multi-national cohort of more than 250 000 petroleum workers. Regul Toxicol Pharmacol 26, 188, 1997.

Wong O. and Raabe G.K. Critical review of cancer epidemiology in petroleum industry employees, with a quantitative meta-analysis by cancer site. Am J Ind Med 15, 283-310, 1989.

Wurster-Hill D.H., McIntyre O.R. and Cornwell G. Marker chromosome 14 in myeloma and plasma cell leukaemia. Lancet 2, 1031, 1973.

Xu F., Gardener A., Tu Y., Michl P., Prager D. and Lichtenstein A. Multiple myeloma cells are protected against dexamethasone induced apoptosis by insulin-like growth factors. Br J Haematol 97, 429-40, 1997.

Yang E. and Korsmeyer S.J. Molecular thanatopsis: A discourse on the bcl-2 family and cell death. Blood 88, 386, 1996.

Yarchoan R., Redfield R.R. and Border S. Mechanisms of B-cell activation in patients with acquired immunodeficiency syndrome and related disorders: contribution of antibody-producing B-cells and of immunoglobulin production induced by human T cell lymphotropic virus type III / lymphadenopathy-associated virus. J Clin Invest 78, 439-47, 1986.

Yip M.Y., Sharma P., Mansberg R. and Lam-Po-Tang P.R.L. Ring chromosomes in hypodiploid and hypotetraploid clones from an elderly patient with plasma cell leukemia. Cancer Genet Cytogenet 47, 47-53, 1990.

Zahm S.H., Blair A. and Weisenburger D.D. Sex differences in the risk of multiple myeloma associated with agriculture. Br J Ind Med 49, 815-6, 1992.

Zandecki M., Obein V., Bernadi F., Soenen V., Flactif M. and Lai J.L. et al. Monoclonal gammopathy of undetermined significance: Chromosome changes are a common finding within bone marrow plasma cells. Br J Haematol 90, 693, 1995.

Zandecki M., Bernadi F., Lai J.L., Facon T., Izydorczyk V., Bauters F. and Cosson A. Image analysis in multiple myeloma at diagnosis. Correlation with cytogenetic study. Cancer Genet Cytogenet 74, 115-19, 1994.

Zaraisky M.I. and Podoltseva E.I. The role of HLA antigens in predicting the active course of multiple myeloma. Ter Arkh 70, 42-6, 1998.

Zawadski Z.A. and Edwards G.A. Dysimmunoglobulinemia in the absence of clinical features of multiple myeloma and macroglobulinemia. Am J Med 42, 67, 1967.

Zbigniew A., Zawadski M.D. and Edwards G. M-components in immunoproliferative disorders. Am J Clin Pathol 48, 418-30, 1967.

Zheng T., Mayne S.T.and Flannery J. The time trends of multiple myeloma in Connecticut, 1935-1987 (letter). Int J Cancer 50, 163-4, 1992.

APPENDIX A

BARAGWANATH HOSPITAL

CASE - CONTROL STUDY ON MULTIPLE MYELOMA

INTERVIEW QUESTIONNAIRE

1. INTERVIEW DATA

1.1 Interviewer: Name .....

1.2 Date of interview .....

1.3 Hospital name and address

.....

.....

.....

1.4 Hospital ward no. .....

2. PATIENT DETAILS

2.1 First name .....

2.2 Surname .....

2.3 Hospital number .....

2.4 Present address .....

.....

.....

Telephone no. .....

2.5 Sex .....

2.6 Ethnic group .....

2.7 Date of birth .....

2.8 Age .....

2.9 Home language and tribe .....

.....

2.10 Marital Status - Married..... Single .....

Divorced..... Widowed.....

3. SOCIOECONOMIC STATUS

3.1 Occupation (see 9.) .....

3.2 Occupational rank (classify into one of the categories below using a standard list).

I Higher professional and administrative occupations

II Lesser professional occupations and employers of industry and retail trades

III Skilled occupations. N, non - manual. M, manual

IV Partly skilled occupations

V Unskilled occupations

IV Unemployed

Other .....

3.3 Years of education\ or highest standard passed

1 - 4 ..... 5 - 8 .....

8 - 12 ..... 13 + ...

3.4 Highest income (per month) Income = nett income

R 0 - 300 ..... R 301 - 600 .....

R 601 - 1000 ..... R 1001 - 1500 .....

R 1501 - 2000 ..... R 2000 + ...

3.5 Current income

R 0 - 300 ..... R 301 - 600 .....

R 601 - 1000 ..... R 1001 - 1500 .....

R 1501 - 2000 ..... R 2000 + ...

3.6 Home ownership

Yes ..... No .....

Where do you own a home? (town\city\village etc.)

.....

3.7 Type of dwelling

House ..... Apartment\Flat .....

Hostel ..... Shack .....

Outbuilding ..... Boarding .....

Other.....

Number of rooms .....

Number of persons\room .....

Rooms = bedrooms or any room used for sleeping

4. SMOKING AND ALCOHOL HABITS

4.1 Do you smoke? yes..... no.....

If yes, go to question 4.7

If no, carry on here

4.2 Have you ever smoked as much as one cigarette per day for a year?

Yes..... no.....

If no, go to question 4.11

If yes, carry on here

Ex - smokers

4.3 What did you smoke?

commercial cigarettes.....

hand - rolled cigarettes.....

pipe.....

4.4 How old were you when you when you started smoking?

.....

4.5 How old were you when you stopped smoking? .....

4.6 Number of years that you were a smoker? .....

4.8 In the past, on average, how much did you smoke per day?

commercial cigarettes.....

hand - rolled cigarettes.....

(Number of cigarettes smoked\day)

1 = 1 - 4 2 = 5 - 9

3 = 10 - 14 4 = >15

Grams of pipe tobacco.....

Go to question 5 now.

Present smokers

4.9 What do you smoke?

commercial cigarettes.....

hand - rolled cigarettes.....

pipe.....

4.10 How old were you when you started smoking? .....

4.11 Number of years smoked upto presently? .....

4.12 How much do you smoke per day at present?

commercial cigarettes.....

hand - rolled cigarettes.....

pipe.....

4.13 In the past, on average, how much did you smoke per day?

commercial cigarettes.....

hand - rolled cigarettes.....

Grams of pipe tobacco.....

ALCOHOL

4.14 Do you drink alcoholic beverages? yes..... no.....

If yes, go to question 4.16

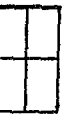
If no, carry on here

4.15 Did you ever drink alcoholic beverages? yes..... no..... 

If no, go to question 5

If yes, carry on here

Ex - drinker

4.16 How old were you when you started drinking? ..... 

4.17 How old were you when you stopped drinking? .....

4.18 Number of years that you consumed alcoholic beverages? 

.....

4.19 In the past, on average, what type of beverage, how much and how often did you drink?

Type of beverage - homebrew\beer\spirits\whisky\gin, 

etc., ...

Amount consumed (per day\week\month) .....

Duration .....

Present drinkers

4.20 How old were you when you started drinking? ..... 

4.21 Number of years that you have drunk alcoholic beverages upto presently? ..... 

4.22 Presently, what type of beverage, how much and how often do you drink?

Type of beverage - homebrew\beer\spirits\whisky\gin, 

etc., ...

Amount consumed (per day\week\month) .....

Duration .....

5. FAMILY HISTORY

5.1 How many members are there in your immediate (parents,spouse,siblings and offspring) family?

.....

5.2 How many members live with you? .....

5.3 Do any of the above members have a chronic illness? ....

If yes, what is the name of the illness?

.....

5.4 Is there any illness which runs in your family?

.....

If yes, what is the illness?

.....

5.5 Does any member of your family have an illness similar to yours, or does any member have cancer?

If yes, explain .....

.....

5.6 Are you in contact with a family member who has a chronic illness?

If yes, what is the nature of the contact and what illness does he\she have?

.....

.....

6. GEOGRAPHICAL HISTORY

6.1 Where were you born?

Town\Township\Village .....

Magisterial District .....

Province (or country, if not RSA).....

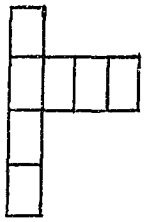
6.2 Where did you spend most of your childhood?

Town\Township\Village .....

Magisterial District .....

Province (or country, if not RSA) .....

From 19___ to 19___ ..... years



6.3 Where else did you stay for six months or longer in the last 10 years?

Town\Township\Village .....

Magisterial District .....

Province (or country, if not RSA) .....

From 19___ to 19___ ..... years

.....

.....

.....

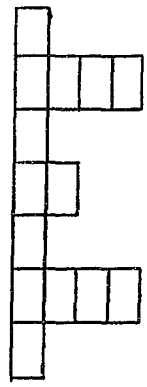
.....

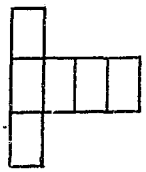
.....

.....

.....

.....





6.4 How far do you live from your present treatment centre?

.....Km



7. PAST MEDICAL AND SURGICAL HISTORY

Medical History

7.1 Do you \ did you suffer from any chronic medical illness?

Yes ..... No .....



If yes, list illnesses and duration of illness

illness\es .....

.....

duration .....

.....

7.2 Have you been immunized? yes.... no....

7.21 Did you receive any of the following immunizations?

	Yes(Y)	No(N)	Unknown (U\K)
Small pox	.....	.....	.....
BCG	.....	.....	.....
Polio	.....	.....	.....
Diphtheria	.....	.....	.....
Typhoid	.....	.....	.....
Whooping Cough	.....	.....	.....
Measles	.....	.....	.....

7.3 During childhood or adult life, did you suffer from any of the following illnesses? (You may have to explain the terminology in simple words or you may have to rely on physical signs which provide clues to some of the illnesses mentioned. Where doubtful, tick the unknown category).

	Y	N	U\K	AGE
Chicken pox	....	....	....	....
Mumps	....	....	....	....
Measles	....	....	....	....
German Measles (Rubella)	....	....	....	....
Shingles (Herpes Zoster)	....	....	....	....
Whooping Cough	....	....	....	....

Tuberculosis ....

Rheumatic Fever ....

Chronic Bronchitis ....

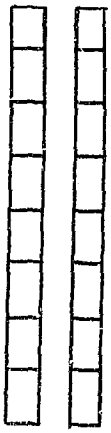
Chronic Otitis media ....

Recurrent Urinary Infections.... ....

Malaria ....

Rheumatoid Arthritis ....

Systemic lupus erythematosus.... ....



Other Auto-immune and chronic inflammatory disorders (eg. scleroderma, Sjogren's disease, dermatomyositis, pernicious anaemia, ulcerative colitis etc.)

....

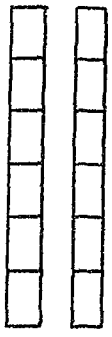
Asthma ....

Eczema ....

Hay fever\allergic rhinitis ....

Skin allergy ....

Any allergy ....

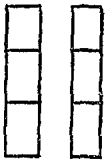


Other illnesses

_____ ....

_____ ....

_____ ....



Surgical History

7.4 Did you have any surgical operations? yes..... no.....



If yes, list name and year of operation .....

.....

.....



DRUG HISTORY

B.1 In the last ten years, did you \ do you take any medication on a chronic or regular basis? yes..... no. ☐

If yes, list names of drugs, frequency of intake,duration and average dose (where possible).

.....

.....

.....

.....

B.2 Do you take any of the following medications on a regular basis?

	Y	N	
Diphenylhydantoins (eg. phenytoin)	.....	.....	☐
Phenobarbitone	.....	.....	☐
Benzodiazepines (eg. diazepam)	.....	.....	☐
Beta blockers (eg. propranolol)	.....	.....	☐
Nitroglycerines	.....	.....	☐
Ibuprofen	.....	.....	☐
Appetite suppressants (diet drugs)	.....	.....	☐

Laxatives (specify)

_____	.....	.....	☐
_____	.....	.....	☐
_____	.....	.....	☐

Herbal \ Traditional medicines (specify)

_____	.....	.....	☐
_____	.....	.....	☐
_____	.....	.....	☐

Cytotoxics (prior to present illness) (specify)

_____ ....

_____ ....

_____ ....

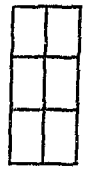


Other medications taken on a regular or chronic basis

_____

_____

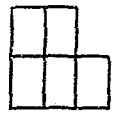
_____



8.3 If you are unsure of the names of the medication you are taking, do you know why the medication has been prescribed (to treat what ailment or disease) ?

.....

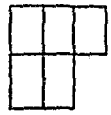
.....

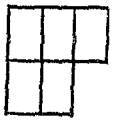


9. OCCUPATIONAL HISTORY

Please start with your first job and finish with your last. We need information about the type of work you did, how long you worked at that job, and whether you were exposed to any substances (eg. chemicals, dust etc.) while doing it.

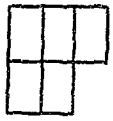
9.1	JOB TITLE	EMPLOYER	WORK DONE (Brief description of work and work place)	DATES 19__-19__	YEARS OR MONTHS WORKED
i)	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....



ii) .... 

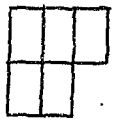
....

....

iii)..... .... 

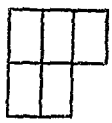
....

....

iv) .... 

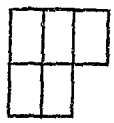
....

....

v) .... 

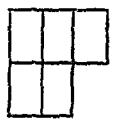
....

....

vi) .... 

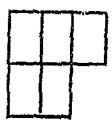
....

....

vii)..... .... 

....

....

viii)..... .... 

....

....

9.2 Radiation exposure

9.21 Did you ever work with X rays? yes..... no.....u\k- 

unknown.....

a) In which year did you first work with the X rays? 

.....

b) For how many years did you work with the X rays?

.....

9.22 Did you ever work with radioactive material?

yes ..... no ..... u/k .....

(a) What was the radioactive material?

.....

(b) In which year did you first work with it?

.....

(c) For how many years did you work with the radioactive material? .....

(d) Where were you exposed to the radioactive material?

.....

9.23 Where you ever treated with radiotherapy (X ray treatment)?

yes ..... no ..... u/k .....

(a) For what were you treated?

.....

(b) For how long did you receive treatment?

.....

(c) When were you treated? .....

(d) Where were you treated? .....

9.24 Did you ever have X rays to any parts of your body?

yes ..... no ..... u/k .....

If yes, how many times to the following areas (1 = 0, 2 = 1 - 4, 3 = >5)

i) Chest .....

ii) Trunk .....

iii) Limbs .....

iv) Other .....

9.3 Farming and agriculture

9.31 Did you ever spend time at or near a farm?

.....

If yes, in what capacity? Owner .....

Labourer .....

Other .....

9.32 What type of farm did you work at ? (e.g. dairy farm, sheep farm,poultry farm, livestock, grain, etc.)

.....

9.33 Describe what you did at the farm (e.g. herdsman, worked in the fields etc.)

.....

.....

9.34 How long did you work \ live at the farm ? 19__ to 19__

9.35 During your stay were you exposed to pesticides (herbicides - eg. phenoxyacid herbicides and chlorophenols., and insecticides - eg. organophosphates, carbamates, dinitroanilines etc.,)

yes ..... no..... u\k .....

If yes, explain .....

.....

9.36 Did you come into contact with live animals or carcasses? If yes, name the type of animals, explain the nature of the contact and the duration of contact with the animals

.....

.....

.....

9.37 What farm equipment did you use\ come into contact with?

..... □

Food processing \ agriculture

9.38 Did you ever work as a \ in

	YES(Y)	NO(N)	UNKNOWN	DURATION
i) Butcher	.....	.....	.....	....
ii) Caterer\cook	.....	.....	.....	.....
iii) Baker\confectioner	.....	.....	.....	.....
iv) Food processor\grocer	.....	.....	.....	.....
v) Forestry industry	.....	.....	.....	.....

Other occupational exposure

	Y	N	U/K	DURATION
9.39 Have you ever been involved in any of the following?	.....	.....	.....	.....
a) Insulation work (heat\electrical)	.....	.....	.....	.....
b) Construction site work	.....	.....	.....	.....
c) Manufacturing asbestos containing articles	.....	.....	.....	.....
d) Working in a power station	.....	.....	.....	.....
e) Manufacturing batteries	.....	.....	.....	.....
f) Working in the plastic industry...	.....	.....	.....	.....
g) Working in the rubber industry....	.....	.....	.....	.....
h) Working with fibreglass\mineral fibres	.....	.....	.....	.....
i) Manufacturing furniture\carpenter				

carpenter's assistant ....

j) Coal Tar ....

k) Welder\welder's assistant ....

l) Printing\paper industry ....

m) Painter\paint manufacturer

spray painter ....

n) Medicinal\pharmaceutical industry. ....

o) Chemicals\dyes ....

p) Benzene\petroleum\solvents ....

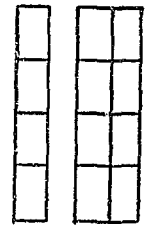
q) Mining (surface\underground) ....

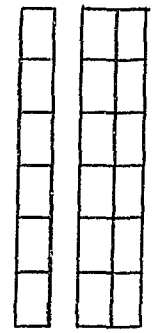
r) Smelter\quarry-man\rockblaster.... ....

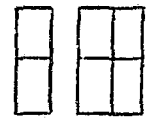
s) Electrician\electrician's

assistant ....

t) Heavy metals e.g. lead ....







10. In your opinion do you think there is any other factor/factors which may have contributed to or precipitated your illness (e.g. stress)

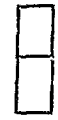
.....

.....

.....

.....

.....



11. GENERAL

The following questions are to be completed by the interviewer only

11.1 Co-operation of subject? good .....

fair .....

poor .....



11.2 Additional comments .....

.....

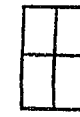
.....

.....



11.3 Language of the interview .....

11.4 Home language of the interviewer .....



Author Patel M
Name of thesis An Epidemiological Study Of Multiple Myeloma In Southern Africa Patel M 1999

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.