THE SPECTRUM OF SKIN BIOPSY PROVEN CUTANEOUS INVASIVE FUNGAL INFECTIONS IN PATIENTS AT JOHANNESBURG ACADEMIC HOSPITALS (JANUARY 2019 – DECEMBER 2021)

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A research report (in the format of a “submissible” paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg,
in partial fulfilment of the requirements for the degree Master of Medicine (Dermatology)

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Declaration

I, Amanda Visagie, declare that this Research Report (in the format of a "submissible" paper) is my own, unaided work. It is being submitted for the Degree of Master of Medicine in Dermatology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

_____

Signature of candidate

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Contribution of the Candidate to the Paper

Declaration: Student's contribution to the article and agreement of co-authors

I, Amanda Visagie, student number A0070642, declare that this Research Report is my own work and that I contributed significantly towards the research findings presented in the paper intended for publication.

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*Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of her Research Report. In cases where the student is not the first author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for her degree purposes.

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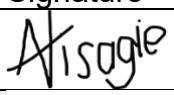
	Amanda Visagie		26/05/2025
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Nil_____

Dedication

To Christ who gives me strength,

to my husband and my daughter, the reason why I pursued this path

to my parents and sister, for years of support and encouragement

to my brother-in-law and Tendesayi Kufa, a thank you for assisting with my raw data.

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List of Abbreviations

AARMS: Academic Affairs and Research Management System
ALT: alanine transferase
ALP: alkaline phosphatase
AST: aspartate transferase
ARV: Antiretroviral
CHBAH: Chris Hani Baragwanath Hospital
CMJAH: Charlotte Maxeke Academic Hospital
FBC: Full blood count
GGT: gamma-glutamyl transferase
Hb: Haemoglobin
HIV: Human Immunodeficiency Virus
HJH: Helen Joseph Hospital
IQR: Interquartile Range
LMIC: Low- and middle-income countries
NHLS: National Health Laboratory Service
PCR: polymerase chain reaction
SA: South Africa
TB: Tuberculosis
VL: Viral load

The spectrum of skin biopsy proven cutaneous invasive fungal infections in patients at Johannesburg academic hospitals (January 2019 – December 2021), South Africa

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Abstract

Background

Fungal infections affect a large percentage of the population, particularly in the setting of HIV (human immunodeficiency virus). It is not well described in low- and middle-income settings. [1] We aimed to describe the aetiology, demographics, and clinical manifestations of invasive fungal infection in Johannesburg, South Africa.

Methods

We conducted a retrospective, descriptive review of skin biopsies done by dermatologists at Johannesburg Academic Hospitals from January 2019 to December 2021.

Biopsies done on patients ≥18 years, reviewed by the Department of Anatomical Pathology, with the diagnosis of invasive fungal infections, were included. Data was obtained from National Health Laboratory Service (NHLS) reports and patient files. Demographics, HIV status, certain serum tests and biopsy results was collected.

Results

60 patients were included with a median age was 40.8 years (median=39; IQR (Interquartile Range) =36-48). The majority of patients were males (66.7%). 46 patients were HIV positive (76.7%). Only 17 (37%) were on ARVs (antiretrovirals). In the HIV positive subgroup, the median CD4 count was 35.5 cells/μL (IQR 8-208) with a median VL (viral load) of 102 copies/mL (IQR 0-276 000). The head and neck areas were predominantly involved (40%). Papules were the most common type of lesions (43.3%). Cryptococcosis (25.7%) and histoplasmosis/emergomycosis (27.3%) were

the most common infections identified. Anaemia was the most predominant abnormality found on laboratory results (67.9%).

Conclusion

Histoplasmosis/emergomycosis and cryptococcosis in HIV positive patients were the most frequently encountered invasive fungal infections in our study population. Untreated/poorly controlled HIV poses a major risk factor for developing invasive fungal infections.

Words 253

Keywords

Invasive fungal infection, skin disease, fungi, Huma Immunodeficiency Virus, immunosuppression, demography, South Africa

Conflicts of interest

None

Introduction

Fungi are ubiquitous organisms that only occasionally cause disease. The most common modes of infection are through inhalation or direct inoculation. Invasive fungal infections affect our population worldwide. In their January 2024 article, Denning et al reported an estimated annual incidence of 6.5 million invasive fungal infections worldwide.[2] There are a number of fungi that can cause cutaneous disease, either alone (often via direct inoculation) or as part of a disseminated process, particularly in immunocompromised patients. These include aspergillosis, blastomycosis, cryptococcosis, chromoblastomycosis, histoplasmosis, mycetoma, emergomyces, sporotrichosis, talaromycosis, zygomycosis [4]. Denning et al reported in their literature review that histoplasmosis and aspergillosis were most frequently diagnosed in their setting in Bangladesh.[2] In their 1997 article of invasive fungal infections at oral sites, Scully et al had similar data, where cryptococcosis and histoplasmosis were diagnosed most frequently.[3] In South-Africa, van Schalkwyk et al, determined in their 2020 article that the most prevalent fungi found in an Academic Hospital in Johannesburg, were to be histoplasmosis/emergomycosis, cryptococcosis and sporotrichosis.[4]

The host's immune system also determines whether inhalation or inoculation will lead to disease or not. Patients with HIV, malignancies, immunosuppressing medications, post-transplant patients and vulnerable patients like neonates and the elderly, are all at higher risk of fungal diseases. [5,6] The morbidity and mortality attributable to fungal diseases have risen compared to previous decades due to the increasing number of immunosuppressed patients and resistance to antifungal treatment. [5]

Cutaneous involvement from fungal infections can include papules, pustules, nodules, plaques, and/or crusting. Furthermore, they can also affect internal organs such as the lungs, liver and lymph nodes. [1]

The site of cutaneous involvement is largely determined by the causative fungus. For example mycetoma and chromoblastomycosis are most frequently seen on the lower limbs whereas cryptococcosis and emergomycosis are most frequently seen at facial and oropharyngeal sites.[3,7–9]

Currently, there is a paucity in literature and research regarding the exact impact that invasive fungal infections have on morbidity and mortality in our setting. There is also little data available on the burden of invasive fungal infections on resource limited healthcare facilities. Another challenge is the difficulty in identifying the exact aetiological fungus.[10]

With this research, our aim was to determine the aetiological agents, patient demographics, and clinical manifestations of invasive fungal cutaneous infection (if a patient had no cutaneous findings, but had a systemic fungal infection, they were not included in the study; only patients with cutaneous findings were evaluated) in a high HIV burden setting in Johannesburg, South Africa.

Materials and Methods

A retrospective, descriptive review was performed on the information obtained from skin biopsies done at the dermatology clinics of Johannesburg Academic Hospitals, including Charlotte Maxeke Academic Hospital (CMJAH), Chris Hani Baragwanath Hospital (CHBAH) and Helen Joseph Hospital (HJH). The study period encompassed a three year period from 01 January 2019 to 31 December 2021.

Data collection:

Dermatologists (both registrars rotating at the different academic hospitals and consultants at these hospitals) are involved with the management of various out- and in-patients with skin conditions. Because the morphology of cutaneous invasive fungal infections are so vast, it is regarded with a high index of suspicion. If there is at all a consideration of a possible fungal infection in both the in-patient and the out-patient setting, a skin punch biopsy will be performed to either confirm or rule out the diagnosis. It is also important to note that the department of dermatology get consulted from various departments at these academic hospital, as well as surrounding local clinics, primary and secondary healthcare facilities. These patients will get reviewed by the dermatologists and biopsies are often performed where indicated.

All biopsies performed on patients 18 years and older by the Department of Dermatology that were reviewed by the Department of Anatomical Pathology with the diagnosis of invasive fungal infections were included in this study. Data were obtained from both National Health Laboratory Service (NHLS) histology reports and clinical patient files at the respective clinics. Each patient's gender, age, HIV status (including

CD4, viral load and treatment status where available) and other comorbidities were also included. This information was entered on a numbered data capturing sheet to ensure anonymity of the patients.

Other information that was collected included the site of the biopsy and clinical description of the lesions. This information was obtained from the Department of Anatomical Pathology (University of Witwatersrand) using the National Health Laboratory Services. Additional blood tests performed at the same time as the biopsy that were also evaluated, included renal function, full blood count and liver function.

Data analysis

Descriptive statistics including counts and percentages were used to describe the study participants with respect to categorical variables such as gender, and the hospital where care was received, while means and standard deviations or medians and interquartile ranges were used to describe continuous variables like age, haematology or biochemistry results, depending on whether they were normally distributed.

X^2/Chi-Square testing was used to determine whether there were statistically significant associations between HIV infection and the given aetiologies and morphologies. A p-value of <0.05 was taken as statistically significant.

The exact causative organism was established by a composite of histological features and culture data. Where the diagnosis was uncertain from the reports, a multi-disciplinary team (including a dermatologist, infectious disease specialist and pathologist) evaluated each individual case to determine if the case should be included

in this study. Characteristics evaluated were the presence or absence of deep seated spores and hyphae, the results of fungal cultures, stains done on the specimens and the clinical background history.

Duration of skin lesions where also recorded. Duration was defined as the period from onset of lesions to the time of biopsy.

Results

66 patients were initially identified in the database, but 6 (9.1%) were excluded from the analysis, 2 because they were younger than 18 years and 4 because the diagnosis was unclear or not at cutaneous sites. The remaining 60 patients were included in the analysis.

Demographics (Table 1)

Among the patients diagnosed with invasive cutaneous fungal infections, the mean age was 40.8 years (standard deviation 10.6 years). There was a male predominance, with males representing 66.7% of the study population, while 31.7% were females.

HIV and other co-morbidities (Table 1)

Of the patients with invasive fungal infections, a large percentage were either HIV positive or had another form of immunosuppression. HIV positivity was confirmed in 46 patients (76.7%), of whom only 17 (37%) were on ARVs. In the HIV positive subgroup, the median CD4 count was 35.5 cells/μL (IQR 8-208) with a median viral load (VL) of 102 copies/mL (IQR 0-276,000). Only 3 (5%) of the patients were known to be HIV negative, with the remaining 11 (18.3%) having an unknown HIV status. Being HIV positive, especially when not virologically suppressed, is a major risk factor for invasive fungal infection. This is reflected in table 1 as well as table 4 (where the X^2 p-value were significant/<0,5). Another form of immunosuppression included one patient (1.7%) with cancer (mycosis fungoides). There were also three patients (5.0%) with various forms of TB (Tuberculosis).

Clinical features (Table 2)

1.Site

Analysis revealed a predominance of head, face, scalp and neck involvement with 24 patients (40%) having lesions in this area. Out of these patients 22 patients were HIV positive. Lower limbs lesions were the second most predominant site in 23 patients (38.3%). Other sites included the upper limbs in 18 patients (30%), and eight patients having truncal involvement (13.3%) whereas the groin was involved in only one patient (1.7%). However, in eight patients (13.3%) this information was not available.

In 14 patients (23.3%) multiple sites where involved. The combinations of sites included the face, lips, limbs and trunk. 12 (26%) of the patients were HIV positive.

2.Morphology

As described by the treating dermatologist, the skin lesion morphologies included papules (43.3%), plaques (26.7%), nodules (21.7%), ulcers (13.3%) and macules (6.7%). In 22 (36.7%) of patients multiple morphologies were described. For 15% of the specimens other descriptive terms were used. In patients with papular lesions, all but one, were HIV positive.

3.Duration

Evaluation of the duration of skin lesions revealed the following breakdown: seven patients (11.7%) had their skin lesions for less than one month, 14 (23.3%) for one month to one year, four (6.7%) for more than one year. More than half of the patients (35/66, 58.3%) were however unsure of the duration.

Aetiology (Table 3; Figure1)

Cultures were only rarely performed (n=7; 11.7%), and most diagnoses were made exclusively on the basis of histopathological features. The most common aetiologies identified were Cryptococcus (n=17; 28.3%) and Histoplasmosis/Emergomyces (n=18; 30%). In all but one of these cases, the patient was HIV positive. In addition, Mycetoma was diagnosed in five cases (8.3%), four patients had chromoblastomycosis (6.7%), two patients had sporotrichosis (3.3%) and two patients had aspergillosis (3.3%). The exact fungal aetiology was however unknown in 12 (20%) cases. These cases were confirmed to have characteristic features of invasive fungal infections like spores/hyphae or stained positive for fungal stains performed, but a specific aetiological diagnosis was not possible on histology or culture.

Additional blood results (Table 4)

Blood results were available for approximately half the patients. Liver function tests were frequently abnormal, with an elevated alanine transferase (ALT) in 60%, elevated aspartate transferase (AST) in 78.6%, elevated alkaline phosphatase (ALP) in 58.6% and gamma-glutamyl transferase (GGT) in 71.4% (table 3). Approximately two thirds of patients (67.9%) were anaemic, and nearly a quarter (21.4%) had a low platelet count.

Discussion

Our study showed a strong association (76.7%) between invasive cutaneous fungal infections and an immunocompromised state.[6] This aligns with previous literature. [11] In particular, advanced HIV appeared to account for the vast majority of the disseminated histoplasmosis/emergomycosis and cryptococcosis diagnosed in our study. Of the HIV patients in our study with a CD4 count available, the median was just 36 cells/μL, indicating severe immunocompromise.

Deep fungal infections can have multiple clinical pictures and can be encountered at different sites.[11] Papules and plaques in the head and neck area and on the lower limbs were most frequently encountered in our study, and therefore should prompt investigation. Most of the patients were unsure of the duration of their skin lesions.[12]

Evaluating the specific causative fungus proved to be challenging. Of note in our study was the low rate of fungal cultures sent during biopsy. Since a positive fungal culture can provide a definitive aetiological diagnosis, this practice should be encouraged in addition to histological sampling. Establishing an aetiological diagnosis is essential, as the optimal treatment strategies vary considerably depending on the particular aetiological fungal pathogen. Other confirmatory testing such a PCR (polymerase chain reaction) can be challenging and expensive to access in LMIC (low-and middle-income countries) settings, and can suffer from a low sensitivity if performed off paraffin-fixed histological specimens. [12] Further research and innovation is needed to improve the diagnosis of invasive fungal infections.

The two most prevalent identifiable fungi encountered in our settings were cryptococcosis (n=17, 28.3%) and histoplasmosis/emergomycosis (n=18; 30%). This was similar to the findings of Schwartz et al in their South African study published in 2017. [11] In keeping with this publication and other literature, patients with these fungi had skin lesions (papules and plaques) most frequently in the head and neck area.

Associated blood results for the study population showed that anaemia was particularly prevalent (low haemoglobin [Hb] ranging from a Hb of 5.7g/dL to 11.5g/dL). This can likely be attributed to anaemia of chronic disease, HIV, and/or bone marrow invasion by the fungus. [9,10]

In addition to the lack of microbiological confirmation, additional limitations of the study included missing data in several cases. This included 11 patients (18.3%) where the HIV status were unknown, and blood results were not available for 24 patients (40%). Additionally, since skin biopsies are typically performed by dermatologists only when they are consulted by general medical teams and or when lesions are troubling to the patient, significant possibility of selection bias exists.

Conclusion

Invasive cutaneous fungal infections is a prevalent category of disease encountered in South Africa and in our hospital settings, particularly considering the HIV-endemic nature of the study population. Although many fungi can cause disease in humans, histoplasmosis/ emergomycosis and cryptococcosis are most frequently encountered in our setting.[11] In our setting almost 80% of patients who were biopsied, were HIV positive.[13]

Of note the specific causative fungal species were not able to be determined from many of the biopsy specimens alone. This could be improved through involving a multidisciplinary team including infectious diseases specialists and microbiologists when a fungal aetiology is involved, and by ensuring that a biopsy sample is sent for appropriate microbiological cultures when a fungal aetiology is suspected.

Table 1: Demographic and clinical characteristics of patients with biopsy proven fungal infections at Johannesburg hospitals, (N=60)

Variable	
Age (mean, sd)*	40.8 (10.6)
Age (median, IQR)	39 (36- 48)
Sex (n, %)	
Female	19 (31.7)
Male	40 (66.7)
Missing	1 (1.6)
Hospital (n, %)	
CHBAH	25 (41.7)
CMJAH	20 (33.3)
HJH	15 (25.0)
HIV status (n, %)	
Negative	3 (5.0)
Positive	46 (76.7)
Unknown	11 (18.3)
On ARVs (n, %)	
No	10 (21.7)
Yes	17 (37.0)
Unknown	19 (41.3)
CD4 count available (n, %)	34 (73.9)
CD4 count cells/ml (median, IQR)	35.5 (8- 208)
VL available (n, %)	27 (58.7)
VL copies/ml (median, IQR)	102 (0- 276 000)
Chronic diseases/previous diagnosis (n, %)	39 (65.0)
No	21 (35.0)
Yes	

*age was normally distributed
Sd: Standard devastation
IQR: Interquartile range

Table 2: Site, duration and morphology of skin lesions among patients with biopsy proven fungal infections at Johannesburg hospitals, (N=60)

Characteristic	Frequency (%)
*Site**	
Scalp/Face/ neck	24 (40.0)
Trunk/buttocks	8 (13.3)
Groin	1 (1.7)
Upper limbs	18 (30.0)
Lower limbs	23 (38.3)
Site unknown	8 (13.3)
Multiple sites	14 (23.3)
Duration	
<1 month	7 (11.7)
1 month - 1 year	14 (23.3)
> 1 year	4 (6.7)
Unknown	35 (58.3)
*Morphology**	
Macules	4 (6.7)
Nodules	13 (21.7)
Papules	26 (43.3)
Plaques	16 (26.7)
Ulcers	8 (13.3)
Other	9 (15.0)
Unknown	4 (6.7)
Mixed	22 (36.7)

*total exceeds 100%; # mass (n=2), abscess (n=2), petechial/purpura/haemorrhagic (n=2), tumour (n=1), cyst (n=1), lesions (n=1)

Table 3: Characteristics of fungal infections and association with HIV infection among patients with biopsy proven fungal infections at Johannesburg hospitals, (N=60)

Characteristic	HIV negative (N=3)	HIV positive (N=46)	HIV status unknown (N=11)	X^2 p-value
Site				
Scalp/Face/ neck	0 (0)	22 (47.8)	2 (18.2)	<0.001
Trunk/buttocks	0 (0)	7 (15.2)	1 (9.1)	
Upper limbs	0 (0)	15 (32.6)	3 (27.3)	
Lower limbs	3 (100)	14 (30.4)	6 (54.6)	
Site unknown	0 (0)	7 (15.2)	1 (9.1)	
Multiple sites	0 (0)	12 (26)	2 (18.2)	
Duration				
<1 month	0 (0)	7 (15.2)	0 (0)	
1month - <1 year	0 (0)	12 (26.1)	2 (18.2)	0.003
>= 1 year	1 (33.3)	3 (6.5)	0 (0)	
Unknown	2 (66.7)	24 (52.2)	9 (81.8)	
Morphology				
Macules	0 (0)	4 (8.7)	0 (0)	<0.001
Nodules	0 (0)	9 (19.6)	4 (36.4)	
Papules	0 (0)	25 (54.4)	1 (9.1)	
Plaques	0 (0)	15 (32.6)	1 (9.1)	
Ulcers	0 (0)	5 (10.9)	3 (27.3)	
Other	2 (66.7)	5 (10.9)	2 (18.2)	
Unknown	1 (33.3)	2 (4.4)	1 (9.1)	
Mixed	2 (66.7)	16 (34.8)	4 (36.4)	
Aetiology				
Histoplasmosis/Emergomyces	0 (0)	17 (37)	1 (9.1)	<0.001
Cryptococcosis	0 (0)	16 (34.8)	1 (9.1)	
Eumycetoma	1 (33.3)	1 (2.2)	3 (27.3)	
Aspergillosis	1 (33.3)	0 (0.0)	1 (7.7)	
Chromoblastomycosis	1 (33.3)	2 (4.3)	1 (7.7)	
Sporotrichosis	0 (0.0)	0 (0.0)	2 (14.4)	
Not specified	0 (0.0)	9 (19.6)	3 (27.3)	

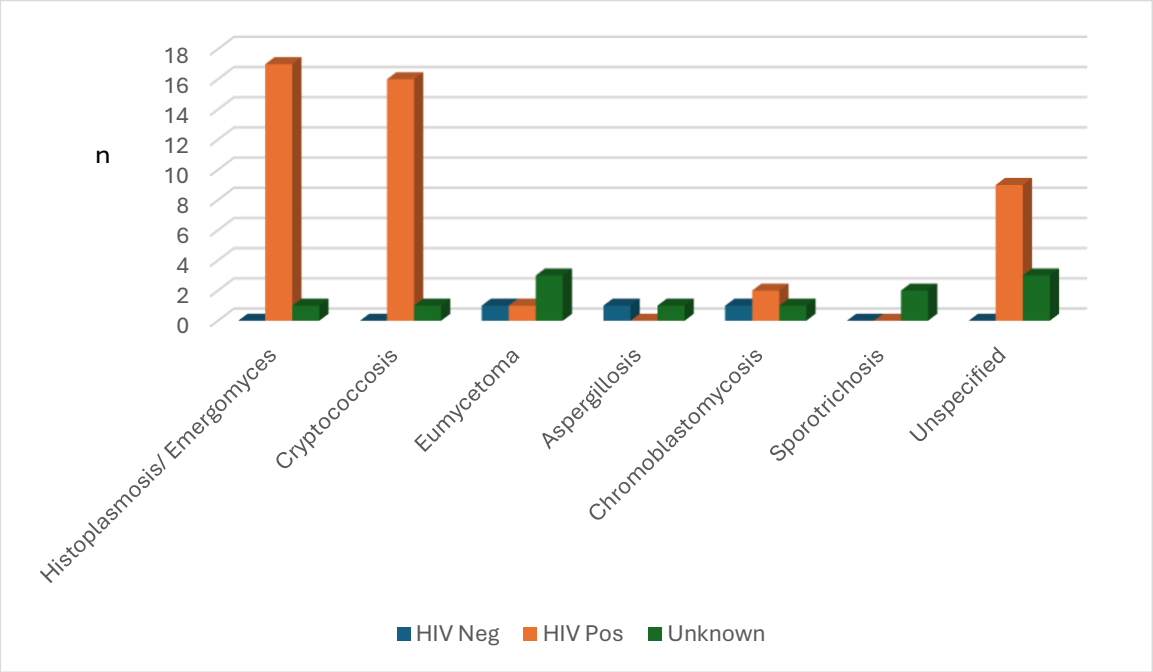
X^2: Chi-squared test

Table 4: Biochemical and haematological results of patients with biopsy proven fungal infections at Johannesburg hospitals, (N=60)

Test	N	Median (IQR)	% <LLN	% >ULN
ALT	30	37.5 (24 - 71)	0/30 (0.0)	18/30 (60.0)
AST	28	57.5 (37.5 - 127.5)	1/28 (3.6)	22/28 (78.6)
ALP	29	125 (84 – 254)	0/29 (0.0)	17/29 (58.6)
GGT	28	80 (39.5 - 137.5)	0/28 (0.0)	20/28 (71.4)
Albumin	30	35.5 (25 - 43)	14/30 (46.7)	0 (0.0)
Urea	31	5.5 (4.0- 8.5)	1/31 (2.6)	8/31 (25.8)
Creatinine	32	85 (67 - 94.5)	1/32 (3.1)	3/32 (9.4)
Sodium	31	138 (136 - 143)	7/31 (22.6)	4/31 (12.9)
Potassium	31	4.6 (4.1 - 5.6)	11/31 (35.5)	3/31 (9.7)
Bicarbonate	29	23 (22 - 26)	10/29 (34.5)	3/29 (10.4)
RCC	28	4.39 (4.02 - 4.89)	6/28 (21.4)	3/28 (10.7)
Hb	28	9.5 (8.95 - 12.95)	19/28 (67.9)	2/28 (7.1)
MCV	28	92.9 (88.15- 96.75)	0/28 (0.0)	3/28 (10.7)
WCC	28	6.76 (5.55 - 8.06)	2/28 (7.1)	2/28 (7.1)
Platelet count	28	292 (224- 403.5)	6/28 (21.4)	5/32 (17.9)

IQR: Interquartile range
LLN: Lower limit of normal
ULN: Upper limit of normal
RCC: Red cell count
Hb: Hemoglobin
MCV: Mean corpuscular volume
WCC: White cell count

Figure 1: Aetiology of invasive fungal infections in patients with biopsy proven fungal infections at Johannesburg hospitals, (N=60)



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Appendix A: Approved research protocol

RESEARCH PROPOSAL

TITLE: THE SPECTRUM OF SKIN BIOPSY PROVEN CUTANEOUS INVASIVE FUNGAL INFECTIONS IN PATIENTS AT JOHANNESBURG ACADEMIC HOSPITALS (JANUARY 2019 – DECEMBER 2021)

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THE SPECTRUM OF SKIN BIOPSY PROVEN INVASIVE CUTANEOUS FUNGAL INFECTIONS IN PATIENTS AT JOHANNESBURG ACADEMIC HOSPITALS

Study Protocol

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1. BACKGROUND AND LITERATURE REVIEW

1.1 General information on invasive fungal infections

Fungi can be found in our direct environment and usually do not cause disease unless inhaled or directly inoculated via an open wound, cut or scratch. The host's immune system also determines whether inhalation or inoculation will lead to disease or not. People with weakened immune systems include patients with cancer, organ transplant patients, HIV positive patients, patients on immunosuppressants, neonates and the elderly. [5] [6]

Invasive fungal infections affect a large percentage of the population worldwide. There are a number of fungi that can cause disease. These include: aspergillosis, blastomycosis, cryptococcosis, chromoblastomycosis, histoplasmosis, mycetoma, emergomyces africanus, sporotrichosis, talaromycosis, zygomycosis, etc. [3] (See table below). The morbidity and mortality has also risen compared to previous decades due to the increasing amounts of immunosuppressed patients and resistance to antifungal treatment. Invasive fungal infections can affect internal organs, the skin primarily or be disseminated. [1]

Mycoses	***Source of spore***	***Endemic areas***
Aspergillosis	Decaying vegetable matter	Ubiquitous
Blastomycosis	Soil	USA Mississippi, Missouri, Ohio. Canada,

		Africa. India. Middle East, Australia
Coccidioidomycosis	Soil	USA south-western. Mexico. Central America
Cryptococcosis	Droppings from pigeons	Ubiquitous
Histoplasmosis	Droppings of birds and bats	USA south-eastern, central and mid- Atlantic states, Puerto Rico. Africa, Latin America, India. Far East, Australia
Paracoccidioidomycosis	Soil?	Latin America, especially Brazil
Zygomycosis	Decaying vegetable matter	Ubiquitous

Reprinted from "Deep mycoses in HIV infection", Scully et al, 1997, Oral diseases, Table 1[3]

Invasive fungal infections present with a wide range of clinical signs, depending on the mode of infection, the specific fungus involved, the immune status of the host and other concomitant diseases. (See table below).[14] Most initial infections are acquired via inhalation of a fungal conidia and present with respiratory symptoms. Van Schalkwyk et al found in their study in 2020 of patients in a hospital in the academic setting of Johannesburg that a lot of patients with deep fungal infections had concomitant tuberculosis. They also found that up to 60% of invasive fungal infections where misdiagnosed or missed at initial diagnosis.

They attributed this to the overlapping respiratory symptoms and the low index of suspicion of invasive fungal infections under clinicians. [4]

System	Condition	Commonly Associated Organisms	Associated Condition or Factor
CNS	Meningitis	*Candida* species	HIV
		Cryptococcus neoformans	HIV
	Abscess	*Aspergillus* species	Hematologic malignancy
		Mucorales fungi	Sinus extension
Sinuses	Sinusitis	*Rhizopus* species	Diabetes mellitus
		Rhizomucor species	Diabetes mellitus
		Mucorales fungi	Diabetes mellitus
		Aspergillus species	Neutropenia
Bone and joint	Osteomyelitis	*Blastomyces dermatitidis*	Concomitant pulmonary infection
		C neoformans	Sarcoidosis, lymphoma
Cardiac	Pericarditis	*Candida* species	Prior thoracic surgery
	Endocarditis	*Candida* species	Indwelling venous catheter
	Myocarditis	*Aspergillus* and *Candida* species	Disseminated disease
Pulmonary	Pneumonia	Angioinvasive *Aspergillus* species	Neutropenia, chronic steroid use
		Candida species	Hospitalization
		Coccidioides immitis	Location in southwest region of United States
		Mucorales fungi	Diabetes mellitus
		B dermatitidis	Location in Great Lakes region of United States
		Histoplasma capsulatum	Location in Mississippi and Ohio River valleys of United States
		C neoformans	Bird feces
		Actinomyces species	Odontogenic infection
		Nocardia species	Alveolar proteinosis
		Pneumocystis jirovecii	HIV
		Candida species	Peritoneal dialysis
Gastrointestinal	Peritonitis	*Candida* species	Hematologic malignancy
	Liver disease	*C neoformans*	Sarcoidosis, lymphoma
		H capsulatum	Location in Mississippi and Ohio River valleys of United States
Genitourinary	Urinary tract infection	*Candida* species	Hospitalization
		Aspergillus species	HIV
		P jirovecii	HIV
	Adrenal disease	*H capsulatum*	Disseminated disease
	Reproductive tract infection	*Actinomyces* species	Intrauterine device

Reprinted from “Imaging spectrum of Invasive Fungal and fungal like infections”, Hilary et al, 2017, Radiographics, Vol 37, Number 4, Page 1121.[14]

Main types of deep fungal infections discussed:

Aspergillosis

Aspergillus, most commonly *A. fumigatus* or *A. flavus,* causes aspergillosis. [7,8] These organisms can be found in air, soil and rotting vegetation. *Aspergillus* cutaneous infections are almost always regarded as nosocomial infections. [15]These rare primary cutaneous aspergillosis can be seen in patients with burns, trauma wounds, surgical wounds, intravenous catheters and macerated skin underlying occlusive dressings. [8] Immunocompromised patients (HIV positive, neutropenic patients (main risk factor), chronic use of corticosteroids, patients on immunosuppressive therapy, patients with graft versus host disease or co-infection with cytomegalovirus) [7] are at an increased risk for disseminated aspergillosis. Inhalation of the fungus can be followed by dissemination to the skin, kidneys, heart and central nervous system. This is associated with a poor prognosis. [8] Clinically necrotic papules, nodules, [8] necrotic ulcers and haemorrhagic bullae [7] can be seen (especially at intravenous catheter sites). Histologically inflammation (suppurative and granulomatous) with or without necrosis can be observed. Aspergillosis shows branching of hyaline, septate hyphae. Staining with silver will demonstrate this well. [8]

Blastomycosis

Blastomyces dermatitidis, one of most common causes of blastomycosis, is a dimorphic fungus that is endemic to North America, Africa and India. [8] [6] Mucocutaneous involvement is a sign of disseminated disease with the patients usually having pulmonary involvement primarily. These patients present with ulceration and papules in their mouth initially, that can worsen to involve the rest of their face. [7] In South Africa other subspecies have been described with the help of

more advanced technology. These subspecies include for example *B. gilchristii, B. parvus, B. percursus, B. emzantsi.* [16] It has also been noted that there are differences between North American blastomycosis and cases described in Africa, as the cases in Africa mostly affect the bone and skin of a patient, whereas those patients from North America mostly have pulmonary involvement. [16] Adults have a higher risk to develop systemic infection with a higher incidence seen in men (likely due to higher exposure to soil in their occupation). Acute pulmonary blastomycosis is mostly seen in children. [8] Primary cutaneous blastomycosis is uncommon but secondary cutaneous dissemination is common. [8] Clinically papules, pustules and verrucous plaques with scaling, central ulceration and crusts can be seen. [17] Healing usually starts in the centre of lesions. Histologically round yeasts with broad based budding and thick double contoured walls can be seen. Pseudoepithelial hyperplasia and inflammation (suppurative and granulomatous) is also seen. Methenamine silver and PAS staining can aid in the diagnosis. [8]

Cryptococcosis

Cryptococcosis is caused by *Cryptococcus species including C. neoformans and C. gattii* [6] which can be found in pigeon droppings. [8] Primary infection cause pulmonary symptoms after inhalation of the fungus, secondary cryptococcosis is described as dissemination to the central nervous system, bone and skin. [7] Cryptococcosis can be found in both immune competent and immunocompromised patients. [18] Dissemination is usually only seen in 10% of cases and these patients usually are immunocompromised patients (especially HIV positive patients) [7]. Clinically cutaneous lesions such as ulcers, cellulitis, molluscum contagiosum-like lesions, papules, nodules and abscesses can be seen in up to 15% of patients with secondary

cryptococcosis.[7,8] Cutaneous lesions is a poor prognostic sign, with a mortality rate as high as 80%. [8] Histologically either gelatinous (numerous organisms, little inflammation) or granulomatous (fewer organisms; more inflammation; little necrosis) findings can be seen. Staining include PAS stain (shows central yeast forms), mucicarmine/ Alcinian blue (shows characteristic capsule) and India ink preparations (shows presence of the capsule). [8]

Chromoblastomycosis

Chromoblastomycosis is usually found in tropical and subtropical climates and at times in the US, Canada and Europe. [8] Farmers, miners and rural workers are at higher risk due to exposure of soil and decaying plants. This likely explains why men between ages of 20 to 60 years of age are more often affected due to increased occupational exposure (90% of cases). [8] Clinically chromoblastomycosis typically presents with a chronic expanding verrucous plaque on the lower or upper extremity. In most cases it affects one of the lower limbs. CNS involvement has also been documented. [7] Histologically pseudoepitheliomatous hyperplasia, intraepidermal abscesses, suppurative and granulomatous inflammation within the dermis are seen. [7,8] A typical finding called copper pennies or Medlar bodies can also be seen in the dermis. These are described as round, pigmented bodies. [8]

Histoplasmosis

The dimorphic fungus *Histoplasma capsulatum* var. *capsulatum* is the cause of histoplasmosis via inhalation or direct cutaneous inoculation. This fungus can be found in soil in warm climates and also caves, construction sites, chicken coops, old buildings and schoolyards. Bats, birds and fowl act as reservoirs for histoplasmosis

and their faeces contain the organisms. [8] In Africa, histoplasmosis is caused by a variant called H. capsulatum var. duboisii. [6] Mucocutaneous, subcutaneous and bone lesions are found in patients with this variant. [8] Clinically histoplasmosis cause either pulmonary disease, primary cutaneous disease or can be found as disseminated histoplasmosis (involving the spleen, lungs, bone marrow lymph nodes, skin and liver) [7]. Cutaneous histoplasmosis is difficult to diagnose due to the skin lesions being non-specific. These lesions include oral ulcers, plaques, and erythematous papules/nodules with a crust or scaling. [19] The extent of these lesions are mostly determined by the patient's immune status, with patients with HIV and low CD4 counts being more likely to have extensive cutaneous involvement. [8] Disseminated histoplasmosis is most often seen in patients with HIV, systemic lupus erythematosus, leukaemia, lymphoma, renal transplantation and patients on corticosteroids. [7] Histologically a rim of clearing can be seen around intracellular yeasts with histiocytes and giant cells also visible. Staining with PAS and Gomori methamine silver can aid in the diagnosis. [8]

Mycetoma

Mycetoma is also called Madura foot or maduromycosis [7]. Mycetoma is found in tropical and subtropical climates in males between the ages of 20 to 50 years old. Three subtypes are described: actinomycotic mycetoma (aerobic and anaerobic organisms, e.g. *Nocardia brasiliensis*, *Actinomadura madurae*), eumycotic mycetoma (fungal) and botryomycosis (bacterial, e.g. *Staphylococcus aureus, Pseudomonas* spp.). [8] Eumycotic mycetoma is often detected in Africa. Direct inoculation of soil into the skin due to trauma the infection. This is usually due to people not wearing protective footwear, patients with malnutrition or immunosuppression and working with

cuts and abrasions exposed to soil [20]. Mycetoma is a granulomatous infection of dermal and subcutaneous tissue. If severe it may involve muscle or bone. [8] Clinically the characteristic triad of draining sinuses, granules and tumefaction, is reported in almost all cases [7]. This usually starts of as a painless papule on the foot, hand, buttock, trunk, arms, head/neck or scalp. [720] Histologically pseudo-epitheliomatous hyperplasia with inflammation and fibrosis of the dermis and subcutis can be seen. [8] Grains of tightly packed colonies of organisms is usually characteristic. To distinguish between the subtypes, special stains like silver/Grocott stains and Brown–Brenn are done. [8]

Sporotrichosis

Sporotrichosis can be found worldwide (especially S. schenckii) but it is most often found in Mexico, Central America, South America and South Africa. Brazil has reported an increase in infection rates in the last decade due to the spread by infected cats (cats harbour a high amount of organisms in ulcers). [8] Individuals who work outdoors like gardeners working with orchids and roses are at higher risk due to direct cutaneous inoculation from thorns or wood. [21] Adults are also at a higher risk, however all age groups have the potential of being infected [7]. Clinically a non-painful papule, ulcer or granulomatous plaque can be seen at the site of inoculation (usually hands). [8] Cutaneous disease (often extensive) with or without systemic involvement is often seen in immunocompromised patients. Involvement of regional lymph nodes has also been described. [7] As mentioned, the response of the host depends on their immune system but it is also affected by the size and virulence of the inoculum. [8] Inhaled sporotrichosis, cutaneous and systemic dissemination has been documented. [8] Histologically the dermis and subcutis shows palisading granulomatous inflammation with stellate suppurative abscesses. [7] Asteroid bodies can be visualised. [8] Cigar-

shaped yeast can be seen with staining with fluorescent-labelled antibodies or PAS, otherwise it can be difficult to visualise the fungi. [8]

Diagnosis of deep fungal infections:

Despite the increase in the patients at risk of fungal diseases (due to immunosuppression, climate change and travel), determining the specific causative fungus in a particular case can be difficult. [6] Depending on the fungus, this may be due to an overlap in histopathological features [21], the lack of a sensitive and specific diagnostic test, a non-specific clinical presentation, a low index of suspicion by clinicians [4], limited access to dermatologists, and scarcity of diagnostic experience. Furthermore, certain fungi that were previously not often the cause of invasive fungal infections, have now emerged as opportunistic pathogens. [6] Unfortunately these factors lead to patients with treatable conditions going untreated or undiagnosed.[11]

Making the right diagnosis timeously is important because of the morbidity and mortality caused by these invasive fungal infections, especially in the setting of disseminated disease. Schwartz et al determined in their study of 2017 in the Western Cape that 50% of patients with systemic mycoses and HIV demised. [11] They also determined that in settings where a diagnosis was made earlier by performing skin biopsies, the outcomes were significantly better.

Diagnostic approaches:

1.Histopathology

One of the methods of diagnosing invasive fungal infections is through histopathological examination (after tissue collection via biopsy) of the affected area/ skin lesions. [22] This is a rapid and cost-effective approach to diagnosis but does have its pitfalls too. Histopathology and staining can be combined with fungal cultures, for a more specific diagnosis, but will yield results quicker than the cultures. [6] Characteristic histological features that aid in the histopathological diagnosis include psuedoepitheliomatous hyperplasia, intraepidermal microabcesses, suppurative granulomatous inflammation, perivascular and interstitial inflammation, small vessel vasculitis, necrosis and fungal elements specific to each specific fungus. [7,8] These features are however not always specific as they can occur in fungi, other infections and non-infectious causes alike. Therefore, special stains can be requested and may help to make the diagnosis. These include Grocott methamine silver and periodic acid-schiff (PAS). These stains can also determine if the infecting organism is intracellular or extracellular and see the presence of a capsule. [22]

STAIN(S)	APPLICATION(S)	COLOR AND FUNGI STAINED
Hematoxylin and eosin (H&E)	Used routinely in pathology to demonstrate tissue morphology; in the case of fungal infections, this stain helps identify the inflammatory host reaction, such as multinucleated giant cells, necrotic material, hemorrhage, and the Splendore-Höeppli phenomenon; most fungi can be observed with this	All fungi show pink cytoplasm, blue nuclei, no colour for the wall

	stain, particularly the nuclei of yeast-like cells or if the fungus is naturally pigmented (however, the fungal elements may be difficult to distinguish from the background)	
Fungal silver stains (Grocott and Gomori methenamine silver [GMS])	They highlight the wall of the fungus and thus are useful for screening the tissue sample, can be combined with H&E in such a way that the fungus and the host reaction can be clearly observed	The fungal cell wall appears black or dark brown for all fungi, the surrounding tissue is usually green, the *Mucorales* may stain very pale
Periodic acid-Schiff (PAS)	Detects glycogen in tissues, fungal walls contain large amounts of glycogen and thus PAS can be used for screening for fungal organisms	The fungal cell wall appears pink to red purple; depending on the counterstain used, the nuclei can be blue
Gridley fungus	Stains the walls of most fungi	The fungal cell wall appears purplish red, the background is usually yellow
Mucin stains (Mayer's or Southgate mucicarmine, Alcian blue)	Stains mucopolysaccharides, including the capsules of a variety of organisms; also stains mucus, which can be present in a variety of human cells	Useful to highlight the capsules of cryptococci, which appear red or blue depending on the stain used
Melanin stains (Fontana-Masson)	Stains melanin present in some fungi; also stains melanin present in human tissues, such as in the skin epidermis	*Cryptococcus* and the dematiaceous fungi will take a black to dark brown color
Bacterial stains (tissue Gram stains or acid-fast stains)	Many fungi take bacterial stains; additionally, some filamentous bacteria (actinomycetes	*Candida* spp. stain purple/blue (Gram positive) with the Gram stain; *Blastomyces* and *Histo*

	and *Nocardia*) have to be differentiated from fungi	*plasma* can be acid-fast staining (take a red color)
Immunohistochemistry (IHC)	Uses antibodies against the different fungi, antibodies can be monoclonal or polyclonal, assays are not FDA approved and require validation by each laboratory	Assays for *Blastomyces*,*Cryptococcus* ,*Histoplasma*,*Coccidioides*, *Pneumocystis*,*Sporothrix*, *Paracoccidioides*,*Penicillium*, *Candida*, *Aspergillus*, and *Mucorales* have been published in the literature; depending on the colorimetric developer, the fungi can stain dark brown or red; the counterstain is usually hematoxylin; cross-reactivity of the antibodies is a concern
In situ hybridization (ISH)	Uses molecular probes for different fungi, probes are generally ribosomal since there are multiple copies of ribosomal genes in each fungal cell, assays are not FDA approved and require validation by each laboratory	Probes published in the literature include those for *Blastomyces*,*Cryptococcus* ,*Histoplasma*, *Coccidioides*, *Pneumocystis*, *Sporothrix*, *Candida*, *Aspergillus*, *Mucorales*, *Fusarium*, and *Pseudallescheria*; depending on the colorimetric developer, the fungi can stain dark brown or red; sensitivity and specificity of the probes vary

Reprinted from "Histopathologic Diagnosis of Fungal Infections in the 21st Century", Gaurner et al, 2011, ASM Journals, Clinical Microbiology Reviews, Vol 24, No 2, Table 5.[6]

2.Other methods

Other ways that aid in making the diagnosis include fungal culture (of skin, or blood/other site), antigen tests (e.g. CrAg, or urine histoplasmosis antigen), antibody tests and molecular tests (PCR, etc.).

-Culture

Fungal cultures can be detected when collected specimens are given a period of time to grow on a agar medium. Making use of cultures can help with the differentiation between different fungi.[23] Unfortunately tissue cultures is not always routinely obtained and may take several weeks for results to be available.[24] Cultures can also be insensitive due to contamination, which makes interpretation difficult. [23]

-Immunohistochemistry

These techniques use antibodies to help with the detection of fungal antigens in thinly sliced tissue that is freshly frozen or embedded in paraffin. These sections can then be treated with enzymes, fluorescence labelled (used with fluorescence microscope) or subjected to antigen retrieval to aid in the viewing and diagnosing of specific fungi. This technique is not costly and can be done relatively quickly. More than one fungus can also be detected at the same time. This unfortunately also means that sometimes a specific diagnosis cannot be met. [6]

-FISH

In situ hybridization utilizing oligonucleotide probes directed against fungal ribosomal RNA are a newer, rapid and accurate method that helps with the identification of

dimorphic fungi in paraffin-embedded tissue. Unfortunately this test is not always available[24].

-PCR

Polymerase chain reaction (PCR) is a test where multiple copies of any DNA from a fungus is extracted and amplified for further evaluation and diagnosis. [6] PCR testing can help tremendously in cases where difficulty in making a specific diagnosis is encountered due to the sensitivity and specificity of PCR testing. The way the tissue is handled and fixed, unfortunately affects the sensitivity. [6] The sensitivity also depends on the amount of fungal elements present in the tissue sample. [6]

3.Methods for diagnosing specific organisms

Morphology using H&E, GMS, and PAS staining	**Suspect fungus(i)**	**Alternative test with nontissue samples**
Broad-based budding yeasts (10–15 micrometre)	*Blastomyces dermatitidis*	-Antigens in urine or serum -Serology -Cultures of sputum or BAL fluid
Narrow-based budding yeasts (4–10 micrometre) with a thick capsule	*Cryptococcus* spp.	-Cryptococcal antigen -Cultures of blood, CSF, and other fluids
Small yeasts (2–4 micrometre) with narrow-based budding grouped in clusters inside macrophages	*Histoplasma capsulatum*	-Antigens in urine or serum -Serology -Cultures of blood and other fluids
Nonpigmented (hyaline), septated hyphae with acute- angle branching	*Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Trichoderma* spp., and *Paecilomyces* spp.	-Detection of galactomannan in serum or BAL fluid -Detection of beta-D-glucan in serum -Cultures of blood

*BAL- Bronchoalveolar lavage

Reprinted from “Histopathologic Diagnosis of Fungal Infections in the 21st Century”, Gaurner et al, 2011, ASM Journals, Clinical Microbiology Reviews, Vol 24, No 2, Table 5.[6]

1.2 The burden of HIV

As already mentioned, HIV (Human Immunodeficiency Virus) is one of the main risk factors seen in most invasive fungal infections. It is therefore important to review the burden of HIV on the cutaneous system and also in South Africa as part of the literature review.

Data analysis from the midyear evaluation from 2020 by Statistics South Africa estimated that the prevalence of HIV in South Africa (SA) is approximately 13,0%. It is estimated that in 2020, 7,8 million people in SA are living with HIV. It was also determined that 18,7% of adults aged 15-49 years in SA are HIV positive. [25]

It is estimated that as many as 45 – 60% of patients hospitalised in medical wards in South Africa are HIV positive. [4] It was also determined that in 2012 – 2013 in an observational study in a hospital in the Western Cape, that 64% of HIV positive patients had a CD4 (cluster of differentiation 4) count below 200 cells/uL. This is despite the fact that ARV’s are readily available and that guidelines stipulate that all HIV positive patients qualify for ARV’s. [13] Low CD4 counts predispose patients to contracting a wide range of infections including invasive fungal infections. These diseases cause a high morbidity and mortality, and also increase the burden on hospitals with higher rates of hospital admissions in often resource-scarce environments. [26]

It was estimated that in HIV positive patients, TB caused 251 000 deaths globally in 2018 and that in 2014 approximately 181 100 people succumbed to cryptococcal meningitis. Similar figures for deep fungal infections are not known due to the paucity of studies in this regard. [27]

Van Schalkwyk et al determined in their 2020 study after reviewing 189 HIV positive in-patients with advanced HIV that, despite access to ARVs, one in ten enrolled patients had evidence of invasive fungal infections. [4]

1.3 Conclusion of literature review

There are a variety of fungi causing invasive fungal infections that have a wide range of clinical presentations which can often be non-specific. This in conjunction with the challenges faced to make the diagnosis of a specific causative fungus, often causes the clinician great difficulty in patient management. Another factor that has a big effect, especially in South Africa, is the considerable effect of HIV on the patient population. The clinical picture of invasive fungal infections in a HIV positive patient may be skewed due to the severity, non-specificity and co-infections with other opportunistic infections. Taking all of this into account, further research and protocols are needed to assist the clinician with swift and accurate diagnosis which will make management more effective. This will also assist in determining the exact burden invasive fungal infections have on the healthcare system and improve overall morbidity and mortality rates.

2. STUDY OBJECTIVES

This study aims to:

1. Describe the sociodemographic characteristics of patients with biopsy proven cutaneous invasive fungal infections that presented with or were followed-up at the Dermatology clinics of the following hospitals:
    - Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)
    - Chris Hani Baragwanath Academic Hospital (CHBAH)
    - Helen Joseph Hospital (HJH)
2. Determine the specific aetiology/fungi causing invasive fungal infections in patients in the above-mentioned facilities. This will be accomplished by reviewing histopathological and mycology data in concert.
3. Describe the spectrum of clinical presentations (morphology of skin lesions) of these invasive fungal infections as per the histology request forms completed by the Dermatology department.
4. Describe the frequency of HIV in this study cohort of patients with skin biopsy proven invasive fungal infections. For all HIV positive patients, the CD4 and viral load (where available) will be recorded.

3. STUDY DESIGN AND METHODS

3.1 Study Design

The study will be a retrospective, descriptive review of skin biopsies done at dermatology clinics at Johannesburg Hospitals (Charlotte Maxeke Academic Hospital, Chris Hani Baragwanath Hospital and Helen Joseph Hospitals). The study period will encompass a three year period (January 2019 – December 2021).

The patients' demographic information, HIV status and biopsy results will be extracted from the histology report. This information will be obtained from the Department of Anatomical Pathology (University of Witwatersrand) using the National Health Laboratory Services. Additional information will be obtained from a review of the patient's blood results at the time of the biopsy.

3.2 Setting

The data will be obtained from the Department of Anatomical Pathology (University of Witwatersrand) using the National Health Laboratory Services. This data is from biopsies done at the following departments and clinics:

- Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)
    - Dermatology Department
- Chris Hani Baragwanath Academic Hospital (CHBAH)
    - Dermatology Department
- Helen Joseph Hospital (HJH)
    - Dermatology Department

OF NOTE: Access to the biopsies done to diagnose invasive fungal infections will be granted by the Department of Anatomical Pathology of the University of Witwatersrand.

3.3 Methods of assessment or measurement

All biopsies done on patients 18 years and older by the Department of Dermatology reviewed by the Department of Anatomical Pathology with the diagnosis of invasive fungal infections will be included in this study. Data will be obtained from both National Health Laboratory Service (NHLS) histology reports and clinical patient files at the respective clinics. All patients will stay anonymous during the course of the study. This information will be entered on a numbered data capturing sheet that will be used to determine the statistics.

3.4 Eligibility criteria

All patients 18 years and older with a histological report indicating an invasive fungal cutaneous infection will be included in the study.

3.5 Exclusion criteria

- If the diagnosis of invasive fungal infections was unclear from the histological report (e.g. a fungal diagnosis “can’t be excluded” or similar).

4. DATA COLLECTION AND STATISTICAL ANALYSIS

4.1 Data collection

With the assistance of the Department of Anatomical Pathology, access to the database of biopsies reported as deep fungal infections will be accessed.

Each patient's data will be captured by means of a data capturing sheet, which will be identified numerically and no use will be made of patients' names. Data will be stored on Microsoft Excel. Information required for each patient and indicated on the data capturing sheet:

- Demographic information
    - Age
    - Gender
- Study number
- HIV status
- CD4 and viral load
- Body site where biopsy was done
- Clinical features of cutaneous manifestation of area where biopsy was done
- Duration of presence of skin lesions
- Predisposing factors for instance the occupation and hobbies of the patient
- The clinical condition of the patient with the review of blood tests done (at the time of/ in close proximity to the time of when the biopsy was done) for instance kidney function, full blood count, liver function, infective markers, cryptococcal antigen, cultures, etc.

- Likely fungal aetiology as per histology report (if indicated) – likely cryptococcus vs likely histoplasmosis/emergomyces vs likely blastomycosis vs likely sporotrichosis vs likely mucormycosis vs other moulds (not defined) vs other yeasts (not defined).
- Other non-fungal aetiological considerations as per requesting dermatologist
- Presence or absence of evidence of disseminated disease
- Other immunocompromising conditions
- Histological characterization: granulomas vs none, yeasts vs hyphae, intracellular vs extracellular vs both, pseudoepitheliomatous hyperplasia and intraepidermal microabscesses.

4.2 Statistical methodology and analysis

Objective 1:

The demographic variables that will be evaluated are age and gender. Age is a continuous numerical variable for which a mean and standard deviation will be determined if the data is normally distributed. If the data is not normally distributed, the median and interquartile range will be determined. Gender is a nominal categorical variable for which the frequency and percentage will be determined for each.

Objective 2-4:

The different types of fungi, clinical morphological description of lesions and HIV status are all nominal categorical variables for which frequency and percentage will be determined for each. The CD4 and viral load are continuous variables, the mean and standard deviation will be determined for normally distributed data, whereas the median and interquartile range will be used if the data is not normally distributed.

The above mentioned information will be presented in tabulated form as well as graphs and pie-charts.

5. STUDY LIMITATIONS

- Histological diagnosis of deep fungal infections may still be inconclusive
- Histology form not filled in correctly

Each case will be reviewed with reference to the histopathological and clinical findings by an expert panel consisting of a histopathologist, a dermatologist and an infectious diseases specialist. These cases will be categorised as likely/definite fungus (histoplasmosis, emergomycosis, cryptococcosis, blastomycosis, sporotrichosis), other deep fungus (specified), indeterminate yeast or mould.

6. ETHICS

The protocol will be submitted to the Human Research Ethics Committee of the University of Witwatersrand for evaluation and approval. All patient data will be captured anonymously and only the relevant clinical information as indicated on the data capturing sheet will be used. Permission to do the study will be obtained from all the relevant parties. The data will be kept on password-protected files and not shared with anyone other than the investigators.

7. FUNDING

Self-funded

8. TIMING

See appendix A: Gantt chart indicating the timing of each component of the study.

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10. APPENDICES

Appendix A: Gantt chart indicating the timing of each component of the study

Appendix B: Data capturing sheet

APPENDIX A: GANTT CHART

GANTT CHART: Expected timing for each component of study

2022	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
Literature review													
Preparing protocol													
Protocol assessment													
Protocol editing													
2023	**DECEMBER**	**JANUARY**	**FEBRUARY**	**MARCH**	**APRIL**	**MAY**	**JUNE**	**JULY**	**AUGUST**	**SEPTEMBER**	**OCTOBER**	**NOVEMBER**	**DECEMBER**
Protocol editing													
Ethics application													
Collecting data													
Data analysis													
Writing up paper & submission													

*Yellow: maternity leave

APPENDIX B: DATA CAPTURING SHEET

Hospital:

Study number:

DEMOGRAPHIC DATA			
	Age		
	Gender		
CLINICAL DATA			
	HIV status		
	CD4 count		
	Viral load		
	ARV: Y/N? Duration?		
	Other diseases		
OTHER SEROLOGY			
	U&E		
	FBC		
	LFT		
	CRP		
	CrAg		
	Cultures		
	Other		
PREDISPOSING FACTORS			
	Hobbies		
	Occupation		
	Other		
SKIN LESION DATA			
	Site of biopsy		
	Duration		
	Morphology:		
	Papule		
	Nodule		
	Plaque		
	Ulcer		
	Abscess		
	Cellulitis		
	Other		
	Polymorphic Y/N		
	Other		
HISTOLOGICAL DATA			
	Psuedoepitheliomatous hyperplasia		

	Suppurative granulomatous inflammation		
	Intraepidermal microabcesses		
	Fungal elements		
	Stains		
TISSUE CULTURE			
DISSEMINATED DISEASE			
FINAL DIAGNOSIS	Likely/definite vs other vs indeterminate		

KEY:

-Likely fungal aetiology as per histology report (if indicated):

1.likely cryptococcus : C

2.likely histoplasmosis/emergomyces : H

3.likely blastomycosis : B

4.likely sporotrichosis : S

5.likely mucormycosis : M

6.other moulds (not defined) : OM

7.other yeasts (not defined). : OY

-Other non-fungal aetiological considerations as per requesting dermatologist

Appendix B: Approval from NHLS

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

10 August 2023

Applicant: Amanda Visagie
Institution: University of the Witwatersrand
E-mail Address: amanda.vanderwalt@gmail.com
Tel: 011 488 4911 **Cell:** 082 367 9059

Project Title: THE SPECTRUM OF SKIN BIOPSY PROVEN CUTANEOUS INVASIVE FUNGAL INFECTIONS IN PATIENTS AT JOHANNESBURG ACADEMIC HOSPITALS (JANUARY 2019 – DECEMBER 2021)
Reference Number: PR2345586

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix C: Approval from Faculty of Health Sciences

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

06 February 2023
Person No: 2528634
PAG

Dr A Visagie
P O Box 61704
Vaalpark
1948
South Africa

Dear Dr Amanda Visagie

Master of Medicine in Dermatology: Approval of Title

We have pleasure in advising that your proposal entitled *The spectrum of skin biopsy proven invasive cutaneous fungal infections in patients at Johannesburg Academic Hospitals* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix D: Approval from HREC/ Ethical clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr A Visagie
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine - Dermatology
Medical School
University

E-mail: amanda.vanderwalt@gmail.com

CC: Supervisor: Drs J Nel, L Pillay and P Swart
<Jeremy.Nel@wits.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2023/08/18

REF: R14/49

PROTOCOL NO: **M230202** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *The spectrum of skin biopsy proven cutaneous invasive fungal infections in patients at Johannesburg Academic Hospitals (January 2019 - December 2021)*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr A Visagie

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230202

NAME: (Principal Investigator)	Dr A Visagie
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine - Dermatology Medical School University
PROJECT TITLE:	*The spectrum of skin biopsy proven cutaneous invasive fungal infections in patients at Johannesburg Academic Hospitals (January 2019 - December 2021)*
DATE CONSIDERED:	2023/02/24
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Drs J Nel, L Pillay and P Swart
APPROVED BY:	Professor P Ruff, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/08/18 **EXPIRY DATE:** 2028/08/17

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

Appendix E: Turn it in report

MMed Amanda Visagie.docx

ORIGINALITY REPORT

8%	7%	5%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	wiredspace.wits.ac.za Internet Source	2%
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Exclude quotes	On	Exclude matches	< 8 words
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Appendix F: Journal author guidelines

International Journal of Dermatology

INTERNATIONAL SOCIETY of DERMATOLOGY

Author Guidelines

IJD SUBMISSION FORMS

Online Submission Contact the Editorial Office

1. ABOUT IJD

Published monthly, the International Journal of Dermatology (IJD) is specifically designed to provide dermatologists around the world with a regular, up-to-date source of information on all aspects of the diagnosis and management of skin diseases.

IJD's longstanding interest in global dermatology and tropical skin diseases began upon its first issue release in the early 1960s. Today a well-established global presence, IJD showcases a comprehensive exploration into all aspects of dermatology as basic sciences, venereology and public health, and in teaching dermatology in developing countries.

IJD is guided by a distinguished, international editorial board and emphasizes a global approach to continuing medical education for physicians and other providers of health care with a specific interest in problems relating to the skin.

2. MANUSCRIPT CATEGORIES

IJD invites the following types of submission:

Review Article IJD welcomes the submission of both narrative reviews and systematic reviews with meta-analysis. Topics may include updates in clinically relevant basic science and cutaneous biology.

The length of the body text should not exceed 5000 words (excluding the abstract, reference list, and figures), with a maximum of 5 figures and tables allowed. These submissions must include an unstructured abstract of a maximum of 250 words. Graphical abstracts, visually summarizing the key message and main results of the submission, are highly encouraged, though optional.

A list of multiple-choice, true or false, and/or open-ended questions may be listed at the end of the article to provide additional educational challenges to the reader. Answers will be made accessible on the Journal's social media platforms. Keywords are a requirement, please see below for further details.

Narrative Review Articles summarize existing knowledge in a particular field without appearing as exhaustive reviews of literature.

Systematic Review Articles with Meta-analysis function as synthesized summaries and critical appraisals that account for explicit questions through detailed and comprehensive literature searches in medicine.

A maximum of 5 supplementary figures/ tables restriction applies to this article type. These will be published under the Supporting Information tab once the article goes live. Tables must be 35 lines or less in portrait layout, with 2.5cm/1-inch margins and 12-point font, to fit on one journal page.

The IJD does not currently consider nonmedical and bibliometric topics for publication under this manuscript type.

Original Article As the primary means of communication, submitted Original Articles should present new data resulting from scientific research employing a broad variety of empirical methods and study protocols.

These major didactic original articles should comprise the following structure: Abstract, Introduction, Materials and Methods, Results, Discussion, and References.

The length should not exceed 3000 words (excluding the abstract, reference list, figure, and table legends), with a maximum of 5 figures and tables and preferably no more than 50 references.

A structured abstract of approximately 250 words must be included and should consist of 4 paragraphs labeled: Background, Methods, Results, and Conclusions. Graphical abstracts, visually summarizing the key message and main results of the submission, are encouraged, though optional.

The authors can also include a Plain-language Summary (maximum 100 words) along with their submission. This supplemental excerpt is not mandatory, but it can enhance the article's visibility and citation while also reaching and benefitting a wider audience of readers. Keywords are a requirement; please see below for further details.

A maximum of 5 supplementary figures/ tables restriction applies to this article type. These will be published under the Supporting Information tab once the article goes live. Tables must be 35 lines or less in portrait layout, with 2.5cm/1-inch margins and 12-point font, to fit on one journal page.

The IJD does not currently consider nonmedical and bibliometric topics for publication under this manuscript type.

Clinicopathological Challenge Photographic essay that includes both a clinical and a pathological photograph in color. This contribution should not exceed 800 words in length, with a maximum of 2-3 figures or tables and 5 references. The submission should include a short unstructured abstract. The article must be formatted according to History, What's the diagnosis? (providing four possibilities), Diagnosis, Discussion and References. Keywords are mandatory.

Short Communication Brief research letter presenting new or preliminary research findings, early reports of therapeutic trials, and/or surveys. This manuscript type should not be subdivided into subheadings in the body of the text, rather, all the required parts (Introduction, Materials & Methods, Results, and Discussion), except for the References, must be given in a single section. The article should not exceed 600 words in length with a maximum

of 5 references and 2 figures/tables. No abstracts and no supplemental materials are permitted.

Correspondence Article: Clinical Correspondence & Letter to the Editor Correspondence Articles should not exceed 600 words in length (excluding title, acknowledgment, and references), with a maximum of 5 references and 2 figures or tables. No abstracts and no supplemental materials are permitted.

Case Reports can be submitted under the Clinical Correspondence manuscript type.

A Clinical Correspondence submission should present a detailed description of the symptoms, signs, diagnosis, treatment, and follow-up of an unusual or novel occurrence. These submissions function as cornerstones of medical progress and provide new ideas and directions in research and medicine.

A Letter to the Editor submission should present or respond to new findings in a brief and concise manner or serve as a short, standalone piece expressing an opinion. It may include a short acknowledgment, 1 or 2 sentences maximum.

The IJD does not currently consider nonmedical and bibliometric topics for publication under this manuscript type.

No mandatory author limit applies to this manuscript type, but a co-authorship of no more than 5 submitters is recommended.

Viewpoint A Viewpoint is a sharply focused critical analysis of a scientific issue of strong current interest within the field of dermatology. Viewpoints may address virtually any important topic relevant to the care of patients with dermatologic conditions or professional matters unique to dermatologists, e.g.: health law and policy, public health, clinical medicine, research and prevention, ethics. Viewpoints should provide differing views on a topic, backed up by literature and accompanied by carefully reasoned arguments and a conclusion. This contribution should not exceed 1200 words, with a maximum of 2-3 tables or figures and no more than 5 references.

On a Human Scale
Articles that relate to social, economic, cultural, artistic and humanitarian aspects of medicine. This contribution should not exceed 1200 words in length, with a maximum of 2 figures or tables and 5 references.
Updates in Medicine
It is not essential that the contribution be heavily referenced but would be very helpful if pertinent and salient references were included, not only for documentation purposes, but also for additional reading. This contribution should not exceed 1200 words in length, with a maximum of 2 figures or tables and 5 references.
Education
Articles about the methodology of curriculum and instruction in dermatology. This contribution should not exceed 1200 words in length, with a maximum of 2 figures or tables and 5 references.
Neglected Tropical Diseases Any type of manuscripts dealing with neglected diseases and special problems encountered by dermatologists working in the tropics. The wordcount and the number of figures or tables may vary depending on manuscript type.

3. SUBMISSION OF MANUSCRIPTS

No Article Processing Charge (APC) applies to authors wanting to publish in the International Journal of Dermatology. Only when authors select the Open Access (OA) option will their submission become subject to an APC.

New submissions should be made via the Research Exchange submission portal https://wiley.atyponrex.com/journal/IJD. You may check the status of your submission at any time by logging on to submission.wiley.com and clicking the “My Submissions” button. For technical help with the submission system, please review our FAQs or contact submissionhelp@wiley.com.

Revised manuscripts must be submitted as revisions as directed by the Research Exchange website. Do not resubmit a revision as a new manuscript as this may result in re-review and considerable delay. The revision should be complete and contain all the tables and figures. Do not resubmit the original manuscript with your revision.

Submission of a manuscript will be held to imply that it contains original unpublished work and is not being submitted for publication elsewhere at the same time. The author must supply a full statement to the Editor about all submissions and previous reports that might be regarded as redundant or duplicate publication of the same or very similar work.

By submitting a manuscript to, or reviewing for, this publication, your name, email address, institutional affiliation, and other contact details the publication might require, will be used for the regular operations of the publication, including, when necessary, sharing with the publisher (Wiley) and partners for production and publication. The publication and the publisher recognize the importance of protecting the personal information collected from users in the operation of these services, and have practices in place to ensure that steps are taken to maintain the security, integrity, and privacy of the personal data collected and processed. You can learn more at https://authorservices.wiley.com/statements/data-protection-policy.html.

Fast-Track Submission IJD offers a fast-track process for a select number of high-priority 'hot topic' manuscripts containing time-sensitive data, e.g.: phase II or III RCTs. This mode of submission grants a first decision within 7 working days and a speedy revision process. Approval for fast track is entirely at the discretion of the Editor. There is no charge for the fast-track service, however, a pre-submission enquiry must be put forward at least 3 weeks before submission. The enquiry should include the abstract of the article and the reason for requesting fast track. To ensure a successful fast-track submission, authors must commit to responding attentively to reviewers' comments and within a timely manner.

Please send all fast-track enquiries to manager@intjderm.org. 4. PREPARATION OF MANUSCRIPTS

Manuscripts must be written in English and must comply with these instructions in every detail.

Text should be supplied in a word-processed format such as Microsoft Word for Windows. Charts and tables are considered textual and should be

supplied in the same format. Figures (illustrations, diagrams, photographs) should be supplied in gif, jpeg, tif or eps format.

All manuscripts must be typed in 12 pt font with lines double spaced and margins of at least 2.5 cm.

Abbreviations must be defined when first used, both in the abstract and in the main text.

Manuscripts must be as succinct as possible. Text must comply with the word and figure limits defined in Section 2. If authors consider that a manuscript should not conform to the limits specified, exceptionally good reasons must be clearly provided in a cover letter accompanying the submission. Repetition of information or data in different sections of the manuscript must be carefully avoided.

Manuscripts should, where appropriate, include:

Title Page The first page of all manuscripts should contain the following information: 1) the title of the paper 2) the full names of each author 3) ORCID number of the author(s) 4) name of the institution(s) at which the research was conducted 5) name, address, telephone number and email address of corresponding author 6) manuscript word count (excluding abstract and references), table and figure count 7) any conflict of interest disclosures (see Section 5) 8) a running head not exceeding 50 characters 9) a list of keywords

Abstracts Authors submitting Original Articles should note that structured abstracts (maximum 250 words) are required. The structured abstract should adopt the format: Background, Methods, Results, Conclusions. In the case of Review Articles and CPC articles, unstructured abstracts must be provided upon submission (maximum 250 words/100 words).

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