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A review of packed red cell transfusions in the neonatal unit at Charlotte Maxeke Johannesburg Academic hospital

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MMED RESEARCH PROJECT



A review of packed red cell transfusions in the neonatal unit at Charlotte Maxeke Johannesburg Academic hospital

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DECLARATION

I, Xiaojun Song, declare that this research report is my own work. I am aware that plagiarism is wrong. I have followed the required conventions in referencing the thoughts and ideas of others. This report is being submitted for the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.



X. Song
On the 7th of June 2017

DEDICATION

I dedicate this work to Marguerite Wessels, my oldest friend and mentor. Thank you for loving God first and always steering me in the right direction. Thank you for always having time for me even in your busy schedule of being an author, editor, lecturer, mother and grandmother.

Lots of love,
Jun

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I would like to express my sincere gratitude to my supervisors, Professor Daynia Ballot and Dr Tanusha Ramdin. Without their continuous encouragement, expert guidance, and incredible patience, this MMed would not have been possible.

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MMED REPORT

A review of packed red cell transfusions in the neonatal unit at Charlotte Maxeke Johannesburg Academic hospital

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Abstract

Background: Packed red cell transfusion (PRT) is a common therapeutic option in neonates with anaemia.

Objectives: The aim of this study is to review the use of PRT in the neonatal unit, and identify any associations with specific complications.

Methods: This is a retrospective descriptive study of all neonates who were admitted to the neonatal unit of Charlotte Maxeke Johannesburg Academic Hospital, younger than 72 hours, from 1 January 2013- 31 December 2014. Neonates older than 72 hours, or with missing data, were excluded from the study. The timing and number of transfusions given to each neonate could not be established with the available data and were therefore not included in this study. Neonates were divided according to weight into 2 groups, those weighing less than 1500g, and those 1500g or larger. Within the groups, neonates who were not transfused were compared with babies who were transfused. By means of univariate and multivariate analysis, the characteristics of the neonates and the incidence of complications were reported.

Results: The study sample included 3432 neonates. Of these, 2395 were ≥1500g and 1037 neonates were < 1500g. The total number of PRT was 435/3432 neonates (12.7%). The incidence of necrotizing enterocolitis (NEC) in the smaller transfused group was 11.9% versus 4.6% in the smaller non-transfused (P<0.001). The incidence of NEC in the larger transfused group was 14.1% versus 0.7% larger non-transfused group (P<0.001). The incidence of late onset sepsis (LOS) in the smaller transfused group was 49.4% versus 12.0% in the smaller non-transfused group (P<0.001). The incidence of LOS was 37.3% in the larger transfused group versus 2.8% in the larger non-transfused group (P<0.001). The incidence of bronchopulmonary dysplasia (BPD) in the smaller transfused group was 50.2% versus 5.5% in the smaller non-transfused group (P<0.001). The incidence of BPD larger transfused group was 21.2% in the group transfused versus 0.5% in the larger non-transfused group (P<0.001). The incidence of intraventricular haemorrhage (IVH) in the smaller transfused group was 22.0% versus 6.1% small non-transfused group (p<0.001). The incidence of IVH in the larger transfused group was 7.0% versus 0.2% in the larger non-transfused group (p<0.001). The incidence of ROP in smaller transfused group was 9.8% versus 2.0% in the smaller non-transfused group (p<0.001). The incidence of conventional mechanical ventilation (CMV) in the smaller transfused group was 40.8% versus 9.1% in the smaller non-transfused group (p<0.001). The incidence of CMV in the larger transfused group was 60.6% versus 8.0% in the larger non-transfused group. The lengths of stay as well as the duration of CMV in the transfused group were more than double the duration of those who were not transfused. In both groups, after multivariate regression analysis, a significant association was found between PRT and CMV, LOS, BPD, IVH and birth weight. NEC and ROP were no longer significantly associated with PRTs.

Conclusion: 12.7% of neonates admitted to CMJAH neonatal unit received a PRT. However, the exact timing and number of PRT per neonate were not known. Very low birth weight neonates are 8 times more likely to be transfused compared to larger babies. Future prospective studies are needed to determine whether PRT cause LOS,

BPD, IVH and longer duration of CMV; and also to determine that NEC is not associated with PRT.

Background

Packed red cell transfusion (PRT) is a common therapeutic option in neonates with anaemia. According to the Neonatal Transfusion International Forum held in 2009, very little is known about how many PRT occur per neonate.[1] The forum proceedings noted that the birth weight of a neonate plays a substantial role in transfusions.[1] Strauss et al 1999, found that 90% of neonates below 1000g would receive a PRT.[2] He also estimated that 38000 premature neonates received more than 300000 PRT annually.[2] According to a survey done by Lin and Hume (2007), the PRT rates were 7-23% over 6000 neonatal admissions, from 5 large neonatal centres.[1]

The decision to transfuse a neonate is usually based on specific haemoglobin (Hb) level and supplemental oxygen requirement. A number of randomized controlled studies have been performed to establish a safer and more evidence based Hb level at which to transfuse. [3,4] Studies utilizing a lower haemoglobin threshold to transfuse are considered restrictive, whereas those utilizing a higher haemoglobin transfusions threshold limit are regarded as liberal. Some studies found no difference between the liberal and restrictive transfusion groups except for a higher number of PRT in the liberal threshold group.[3] Other studies found better neurodevelopmental outcome in the liberal transfusion groups.[4] In recent years, many institutions, including the Charlotte Maxeke Academic Hospital (CMJAH) neonatal unit, have adopted a restrictive approach to reduce the number of PRT and donor exposures.

Packed red cell transfusions are linked to a number of neonatal complications such as retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD) and more recently necrotizing enterocolitis (NEC).[4,5,6,7] ROP may be associated with PRT because anaemic neonates require longer supplemental oxygen and PRT requirements are also the highest in the extremely premature babies, who are also the mostly likely to develop ROP. Some studies have found no difference in the rates of IVH between the restrictive and the liberal transfusion groups. Two large randomized controlled trials were contradictory: The PINT (preterm infant in need of transfusion) study found that there was more PRT in the higher threshold group without any increase in benefits.[5] The Iowa trial found that there were more severe IVH's in the restrictive group.[4] BPD was associated with a larger volume of PRT in some studies whereas other studies found no association between BPD and PRT.[6] Study by Krimmel et al, 2009, showed an association between NEC, feeding and PRT. The study showed that mesenteric blood flow which usually increases following a feed, fails to rise following a blood transfusion and likely causing gastrointestinal injury.[7] Many authors have done studies on the associations between NEC and PRT but none are conclusive owing to the small size of the studies and because of many confounding factors. Newer studies show no association between NEC and PRT.[8]

Numerous PRT occur in the CMJAH neonatal unit, which is similar to other neonatal centres. However, the decision on whether to transfuse remains open, at the discretion of the consultant and much research concerning the complications of PRT is still needed. This study aims to establish the number of transfusions being administered between the 2 groups (VLBW group - <1500g and larger babies ≥1500g). In addition

it seeks to document the rate of complications in the transfused versus non-transfused neonates within the VLBW and larger baby groups.

Methods

This is a retrospective descriptive study of all neonates, who were admitted to the neonatal unit of Charlotte Maxeke Academic Johannesburg, younger than 72 hours, from 1 January 2013- 31 December 2014. Neonates older than 72 hours, or with missing data, were excluded from the study. The timing and number of transfusions given to each neonate could not be established with the available data and were therefore not included in this study. The data of the VLBW group and larger babies are being reviewed. The characteristics of neonates that received packed red cell transfusions (PRT) were reported. Neonates who received PRT were compared to neonates who didn't receive PRT and the clinical significance was calculated. The decision to transfuse packed red cells was based on CMJAH neonatal unit transfusion guidelines as well as the opinions of the attending physicians. Neonates are transfused with 20ml/kg of packed red cells and furosemide is usually administered mid-way through the transfusion.

Database

All neonatal records at CMJAH are captured on REDCap (Research Electronic Data Capture) when they are discharged. Data collected from REDCap neonatal database included the profile of the baby (inborn, age at admission, birth weight, gestational age and gender), as well as the maternal profile (RPR or HIV positive) and the duration of various modes of ventilation (CPAP, CMV and HFOV). Morbidities such as EOS, LOS, IVH, NEC, BPD and ROP were also collected as well as the outcome (survival or death), duration of stay, and discharge weight. The timing and the number of transfusions given to each neonate could not be established with the available data and were unfortunately not included in this study.

Abbreviations

RPR (rapid plasma reagin) is a screening test for syphilis
HIV (Human Immunodeficiency Virus)
CPAP (continuous positive airway pressure)
CMV(conventional mechanical ventilation)
HFOV (high frequency oscillatory ventilation)
IVH (Intraventricular haemorrhage)- only grade 2 or more was collected in the database.
BPD (bronchopulmonary dysplasia)- requiring oxygen at 28 days
ROP (retinopathy of prematurity)
NEC (necrotizing enterocolitis) - only grade 2 or more was collected in the database.
EOS (early onset sepsis) – culture proven sepsis occurring < 3 days old
LOS (late onset sepsis) – culture proven sepsis occurring > 3 days old

Statistical analysis

Data from the neonatal database was initially uploaded into an MS Excel spreadsheet and edited. Variables not pertaining to the study were removed. The age of admission,

duration of CPAP, duration of CMV and duration of HFOV, as well as the duration of the stay was calculated using formulas in the MS Excel spreadsheet. The data was then uploaded into the SPSS version 19, which is a statistical software package. Categorical variables were described using frequencies and percentages.

Continuous variables, with a normal distribution, were described using mean and standard deviations. In order to test whether there was a significant difference between those who were transfused and those who were not transfused, the Chi squared test was used for categorical variables and the cut-off point for the P value was 0.050. An independent t test was used for comparing continuous variables. The data was subjected to a multivariate logistic regression analysis to derive the odds ratio and the 95% confidence interval to adjust for the differences.

Ethics

Human research ethics committee granted ethics approval for the study. Clearance certificate no. M150976.

Results

A total of 3679 neonates were admitted to the Charlotte Maxeke Academic Johannesburg Hospital during the 2-year study period, of which 2395 neonates were larger babies and 1037 were VLBW. As per the exclusion criteria, 221 neonates who were admitted after 72 hours were excluded. There were 16 neonates whose records were missing as well as 10 neonates who were incorrectly classified were also excluded from the study. This resulted in 3432 of 3679 neonates were included for analysis in the study. Over a 2-year period, 435 of the 3432 (12.7%) neonates received one or more packed red cell transfusion. However, one of the limitations of the study was we could not quantify the number of transfusions per neonate.

Table 1a: The frequencies of the following characteristics in the VLBW group

Variable	**VLBW group** N=1037 n=(%)
PRT	336 (32.4)
Inborn	866 (83.4)
RVD exposed	300 (28.9)
RPR exposed	13 (1.3)
Female	552 (53.2)
NCPAP only	617 (59.4)
CMV	201 (19.4)
HFOV	11 (1.1)
EOS	36 (3.5)
LOS	250 (24.1)
BPD	208 (20)
ROP	47 (4.5)
IVH	117 (11.3)
NEC	72 (6.9)
Survived	762 (73.4)
NEC with surgery	19 (1.8)

Table 1b: The frequencies of the following characteristics in the larger babies group.

Variable	Larger babies N=2395 n=(%)
PRT	99 (4.1)
Inborn	1811 (75.6)
RVD exposed	695 (29)
RPR exposed	32 (1.3)
Female	970 (40.5)
NCPAP only	333 (13.9)
CMV	245 (10.2)
HFOV	16 (0.7)
EOS	63 (2.6)
LOS	102 (4.3)
BPD	33 (1.4)
IVH	12 (0.5)
NEC	29 (1.2)
Survived	2276 (95)

In the VLBW group, there was a higher frequency of PRT, ventilation (NCPAP, CMV and HFOV), LOS, BPD, IVH and NEC. In the larger babies, 21.6% more neonates survived compared to the VLBW group.

Table 2: Demographics of those transfused (T/F) and non T/F in VLBW group and larger babies.

Demographic means	VLBW group			Larger babies		
	T/F	Non-T/F	P values	T/F	Non-T/F	P values
Age on admission [days (SD)]	1.0 (0.2)	1.0 (0.2)	0.27	1.3 (0.64)	1.1 (0.42)	<0.001
Gestational age [weeks (SD)]	28.6 (2.8)	29.5 (2.1)	<0.001	35.0 (3.74)	36.7 (3.44)	<0.001
Birth weight [grams (SD)]	1050.9 (256.8)	1153.0 (189.7)	<0.001	2246.4 (642.83)	2646.0 (768.92)	<0.001
Duration of NCPAP [days (SD)]	5.5 (7.7)	3.0 (2.5)	<0.001	3.9 (4.55)	2.5(2.12)	0.002
Duration of CMV [days (SD)]	11.9 (12.3)	4.3 (3.4)	<0.001	14.4 (1.64)	4.6 (0.23)	<0.001
Duration of stay [days (SD)]	46.4 (26.0)	22.8 (16.7)	<0.001	25.9 (2.44)	9.6(0.53)	<0.001

In both the VLBW and larger babies, there was no difference between the age on admission and the gestational age in the transfused and non-transfused groups.

Unfortunately, the age and gestational age at which the neonate was transfused was not available. The duration of NCPAP, CMV and length of stay were longer in the transfused group.

Table 3: Comparison of conditions in the larger babies, with and without transfusion.

Condition	Transfused N=99 n=(%)	Non-transfused N=2296 n=(%)	P value
Inborn	51(51.5)	1760 (76.7)	<0.001
HIV exposed	30(30.3)	665(29.0)	0.774
RPR exposed	3(3.0)	29 (1.2)	0.134
Male	62(62.6)	1361(59.2)	0.608
NCPAP	25(25.2)	308 (13.4)	0.001
CMV	60(60.6)	185(8.0)	<0.001
EOS	7(7.1)	56 (2.4)	0.005
LOS	37(37.3)	65 (2.8)	<0.001
BPD	21(21.2)	12(0.5)	<0.001
IVH	7(7.0)	5 (0.2)	<0.001
NEC	14(14.1)	15(0.7)	<0.001
Survived	79(79.8)	2197(95.8)	<0.001

In the larger babies, NCPAP, CMV, EOS, LOS, BPD, IVH and NEC occurred significantly more in the transfused group. "Survived" occurred significantly more in the non-transfused group possibly because sicker neonates were more likely to be transfused.

Table 4: Logistic regression in the larger babies

Conditions	OR	95% CI*	P value
CMV	0.13	0.07-0.22	<0.001
LOS	0.17	0.09-0.31	<0.001
BPD	0.15	0.05-0.41	<0.001
IVH	0.09	0.02-0.33	<0.001

*rounded off to 2 decimal places

After a multivariate analysis, CMV, LOS, BPD, IVH were still significant in the larger babies who were transfused. The numbers of intraventricular haemorrhages occurring in larger babies were too small to consider significant.

Table 5: Comparison of conditions, in the VLBW group, with and without transfusion.

Condition	Transfused N=336 n=(%)	Non-transfused N=701 n=(%)	P value
Inborn	281(83.6)	585(83.4)	0.485
HIV exposed	98(29.1)	202(28.8)	0.907
RPR exposed	3(0.9)	10(1.4)	0.470
Female	166(49.4)	386(55)	0.209
NCPAP	251(74.7)	366(52.2)	<0.001
CMV	137(40.8)	64(9.1)	<0.001
HFOV	9(2.7)	2(0.2)	<0.001
EOS	9(2.7)	27(3.9)	0.522
LOS	166(49.4)	84(12.0)	<0.001
BPD	169(50.2)	39(5.5)	<0.001
ROP	33(9.8)	14(2.0)	<0.001
IVH	74(22.0)	43(6.1)	<0.001
NEC	40(11.9)	32(4.6)	<0.001
NEC with surgery	14(4.2)	5(0.7)	<0.001
Survived	256(76.2)	507(72.3)	0.323

In the VLBW group, NCPAP, CMV, HFOV, LOS, BPD, ROP, IVH, NEC and NEC with surgery were significantly more in the transfused group.

Table 6: Logistic regression in the VLBW group

Conditions	OR	95% CI *	P value
LOS	0.26	0.09-0.77	0.015
IVH	1.03	1.00-1.06	0.024

*rounded off to 2 decimal places

After the multivariate analysis, LOS and IVH were still significant in the VLBW group who was transfused.

Discussion

This was the first study at the CMJAH neonatal unit to review packed red cell transfusions.

During the 2-year study period, 435 of the 3432, 12.7% neonates in the study received one or more PRT. Due to the retrospective nature of the study it wasn't possible to document the number of transfusions per neonate. The 12.7% is similar to a survey done by Lin and Hume who found that PRT rates were about 7-23%.[1] Strauss et al also estimated that 38000 premature neonates received more than 300000 PRT annually, which is 12.7%, and which is the same as CMJAH's transfusion rate of 12.7%.[2] In this study, more neonates in the VLBW group had a PRT (32.4%) compared to the larger babies (4.1%).

The incidence of NEC in the VLBW transfused group was 11.9% versus 4.6% in the non-transfused ($P<0.001$). The incidence of NEC in the larger babies who were transfused was 14.1% versus 0.7% in the non-transfused group ($P<0.001$). The results show a significant association between NEC and PRT. The association between NEC and PRT, however, was no longer significant after an adjustment for confounding variables in a logistic regression analysis. This is in keeping with recent retrospective studies by Wallenstein et al (2014) and Sharma et al (2014), who found no association between PRT and NEC.[9,10] Other studies found that PRT lowered the incidence of NEC. A case-control study by Al Faleh et al (2014) found a lower incidence of NEC in neonates receiving PRT, and a prospective trial by Patel et al (2016) who found that VLBW neonates with severe anaemia had an increased risk of NEC if they were not transfused.[8,11]

The incidence of LOS in the VLBW group was 49.4% in the transfused group versus 12.0% in the non-transfused group ($P<0.001$).[12]The incidence of LOS in the larger babies was 37.3% in the transfused group versus 2.8% in the non-transfused group ($P<0.001$). The results show a higher incidence of LOS in the neonates receiving PRT. The association between LOS and PRT persisted after a logistic regression analysis. The association between LOS and PRT could be explained by the findings of Jufferman, whose retrospective study reviewed the association between the development of nosocomial bacterial infection and the transfusion of leucodepleted packed red cells.[12] Jufferman et al concluded that PRT are associated with secondary bacterial infection in patients with sepsis, possibly due to immunomodulatory effects of PRT and this contributed to the adverse outcomes in the recovery phase of sepsis. However, since this study did not differentiate between whether the PRT was given before or after the onset of LOS, it is not possible to draw any conclusions regarding this postulate.

The incidence of BPD in the VLBW group was 50.2% in the transfused group versus 5.5% in non-transfused group ($P<0.001$). The incidence of BPD in the larger transfused neonates was 21.2% versus 0.5% in the non-transfused group ($P<0.001$). The association between BPD and PRT persisted only in the larger babies after an adjustment was made to the confounding factors in a logistic regression analysis.

In our study, the temporal relationship between the transfusions is unknown and we are unable to say whether the blood transfusions caused BPD or whether babies with

BPD are more likely to be transfused. The practice in our unit, is that babies with BPD and anaemia are transfused to facilitate their weaning off oxygen.

The incidence of IVH in the VLBW transfused group was 22.0% versus 6.1% in the non-transfused group ($p<0.001$). The incidence of IVH in the larger babies transfused was 7.0% versus 0.2% in the non-transfused group ($p<0.001$). After the adjustment was made with logistic regression analysis, the association between IVH and PRT persisted for both groups. A study by Christensen et al found there was an association between PRT in the first days of life and IVH, however, a causal relationship could not be concluded.[13] It is uncertain whether PRT caused IVH or IVH resulted in a PRT.

The incidence of ROP in VLBW transfused group was 9.8% versus 2.0% in the non-transfused group ($p<0.001$). ROP's association with PRT was no longer significant after the logistic regression analysis. The incidences of ROP in the larger babies were not recorded because it is a disease related to prematurity. Our study found that there was no significant association between ROP and blood transfusions.

The incidence of CMV in the VLBW transfused group was 40.8% versus 9.1% in the non-transfused ($p<0.001$). The incidence of CMV in the larger transfused babies was 60.6% versus 8.0% in the non-transfused group ($p<0.001$). After adjustment was made with logistic regression, the association between CMV and PRT was still significant in the larger babies. The lengths of stay as well as the duration of CMV in the transfused group were more than double the duration of those who were not transfused in both VLBW group and larger babies. The increased duration of both, hospital stay and ventilation, in neonates who were transfused is possibly because those babies who received transfusions are likely to have been more ill and would, therefore, require longer medical care.

This study serves as a starting point for further studies assessing the use of PRT and their role in the development of numerous complications. Any further studies, must collect more detailed information such as the date of transfusion, number of transfusions, haemodynamic parameters at time of transfusions, Hb at which transfused, as well the date of development of complications.

This study demonstrates that PRT is not a benign intervention. Physicians must always bear in mind the potential complications when deciding on whether to transfuse.

Limitations

The study was a retrospective study. The number of transfusions that occurred per patient and the timing of transfusions were not known therefore it was not possible to establish a causal relationship between blood transfusions and complications. Gestational age is not always accurate because routine antenatal ultrasound is not done.

Conclusion

The number of neonates who were admitted to CMJAH neonatal unit receiving a PRT was 12.6%. Very low birth weight neonates are 8 times more likely to be transfused compared to larger babies. In both groups, after multivariate analysis, a significant association was found between PRT and LOS and IVH. NEC and ROP were no longer significant. Further prospective studies will be required to find a causal relationship.

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APPENDIX A: AUTHOR GUIDELINES

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Short items are more likely to appeal to our readers and therefore to be accepted for publication.

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Review articles are rarely accepted unless invited.

Letters to the editor, if intended for the correspondence column, should be marked 'for publication' and signed by all authors. Letters should be no longer than 400 words with only one illustration or table.

Obituaries should not exceed 400 words and may be accompanied by a photograph.

MANUSCRIPT PREPARATION

Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.

Refer to articles in recent issues for guidance on the presentation of headings and subheadings.

Abbreviations should be spelt out when first used in the text and thereafter used consistently.

Scientific measurements should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl.

If in doubt, refer to 'uniform requirements' above.

ILLUSTRATIONS

Figures consist of all material that cannot be set in type, such as photographs and line drawings. If any tables or illustrations submitted have been published elsewhere, the author should obtain written consent to republication from the copyright holder and the author(s). All illustrations, figures etc. must be of high resolution/quality, preferably jpeg or equivalent but not powerpoint, and preferably attached as supplementary files.

REFERENCES

References should be inserted in the text as superior numbers and should be listed at the end of the article in numerical and not in alphabetical order.

Authors are responsible for verification of references from the original sources.

References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given followed by et al. First and last page numbers should be given.

Journal references should appear thus:

Price NC . Importance of asking about glaucoma. BMJ 1983; 286: 349-350.

Book references should be set out as follows:

Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975: 96-101.

Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-472.

Manuscripts accepted but not yet published can be included as references followed by

(in press).

Unpublished observations and personal communications may be cited in the text, but not in the reference list

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4. The text is single-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses). Figures consist of all material that cannot be set in type, such as photographs and line drawings. If any tables or illustrations submitted have been published elsewhere, the author should obtain written consent to republication from the copyright holder and the author(s). All illustrations, figures etc. must be of high resolution/quality, preferably jpeg or equivalent but not powerpoint, and preferably attached as supplementary files.
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APPENDIX B: PROTOCOL

Mmed Paediatrics

Research Protocol

Candidate: Dr. Xiaojun Song MBChB

Student number: 1225577

A review of packed red cell transfusions in the neonatal unit at Charlotte Maxeke Johannesburg Academic hospital

Supervisor: Prof Daynia Ballot, MBBCh, FC Paeds, PhD Med

Co-supervisor: Dr. Tanusha Ramdin, MBBCh, DCH, FC Paeds, Mmed, Neonatology

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4. Appendix A

5. Table 1

Introduction

Packed red cell transfusion (PRT) is a common therapeutic option in neonates with anaemia. The alternatives to PRT, which are being investigated, are delayed umbilical cord clamping and erythropoietin. Packed red cell transfusions has various side effects and is linked to certain neonatal complications such as retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchodysplasia (BPD) and more recently necrotizing enterocolitis (NEC). [9-15] Even though many PRTs are given, the question of when to transfuse a neonate is still mostly opinion based. Transfusion practices differ from institution to institution and even from neonatologist to neonatologist.

According to a Neonatal Transfusion International Forum held in 2009, very little is known about how many PRTs occur per neonate including "*transfusion rates to neonates per 1000 admissions*".[1] It was found at the forum that the birth weight of a neonate plays a substantial role in transfusions. Strauss et al 1999 found that 90% of neonates below 1000g would receive a PRT.[2] According to a survey done by Lin and Hume 2007, of 5 large neonatal centers across Canada including over 6000 neonatal admissions to NICU per year, the transfusion rates of PRTs were about 7-23%.[1] Strauss et al also estimated that 38000 premature neonates receive more than 300000 PRTs annually.[2]

Alternatives to PRTs include delayed umbilical cord clamping at birth and erythropoietin. Niermeyer and Velaphi 2013 found that delayed umbilical cord clamping at birth is beneficial in term and preterm neonates who do not need resuscitation. At birth, delaying umbilical cord clamping until a neonate starts breathing results in a gentler cardiopulmonary transition.[3] Levy and Blickstein 2006, showed that delayed umbilical cord clamping results in a reduction of PRTs for anaemia, IVH, late onset sepsis (possibly secondary to transfusion of progenitor cells) and NEC.[4]

Physiological anaemia is primarily due to a blunted erythropoietin response.[5] Haemoglobin levels, in physiological anaemia, reach a nadir of around 10g/dL in neonates at 3 months of age and are exaggerated in preterm infants, reaching a nadir as low as 6g/dL at 2 months of age.[5] Erythropoietin was initially widely used. Erythropoietin is now no longer recommended due to the increased risk of retinopathy of prematurity and this was shown by a systematic review done by Aher and Ohlsson 2006.[6]

However, the use of PRTs in neonates is not without risks. Possible side effects include infections, adverse leucocyte reactions (febrile reactions, graft versus host disease and lung injury), circulatory overload and hemolytic reactions (ABO incompatibility).[7] Preservatives in the packed red cells can also be harmful. Citrate can result in hypocalcaemia or hypoglycaemia, which can be very harmful to a neonate and may result in seizures and/or death. Ingredients of preservative solutions like mannitol and adenine may result in diuresis or nephrotoxicity respectively. Potassium may also cause arrhythmias. [7]

At the CMJAH blood bank, each adult packed red cell is divided into 3 neonatal packed red cells and neonates are exposed to different donors, as a single donor policy

is not in effect. All neonates, however, are transfused with leucodepleted packed red cells. Each unit of blood is screened for HIV 1 &2, Hepatitis B, C viruses and Syphilis. The use of leucodepleted PRTs in neonates has decreased the contraction of Cytomegalovirus(CMV) as CMV is known to reside in leucocytes. The transfusion reaction risks are minimal due to the mixing of recipient blood with the donor blood to confirm compatibility. Residual risks occur from donations during the time when antigens or antibodies are of undetectable levels.[8]

Studies have shown that BPD, IVH, ROP and NEC have been associated with PRTs.[9-15] Two small studies by Chen et al 2009 and Brooks et al 1999 found no difference in incidence of IVH between restrictive transfusion groups and liberal transfusion groups. However the results from 2 larger randomized controlled trials, Iowa and PINT trials, were contradictory.[9-12] It was found that BPD was associated with larger volume PRTs in some studies and other studies found no association between BPD and PRT.[13,14] Similarly, ROP may be associated to PRTs because anaemic neonates require longer supplemental oxygen and PRT requirements are highest in extreme prematures.[10] The link between NEC and PRTs may be explained by a reduction in mesenteric blood flow after feeding, being suddenly increased after PRT.[15] Many authors have done studies on the above associations but none were conclusive due to the small size of the studies and many confounding factors.

The decision to transfuse a neonate is usually based on specific haemoglobin(Hb) levels and supplemental oxygen requirements. A number of randomized controlled studies have been done on different Hb levels to establish a safer and more evidence based Hb level at which to transfuse. [10-15] Studies with lower Hb thresholds to transfuse were considered restrictive, while liberal transfusion criteria had a higher Hb threshold. Bifano et al 2001 were the first to perform a prospective study on neonatal PRTs. In this study 50 neonates were randomized to two haemocrit groups, >32% and < 30%. There was no difference between morbidity, mortality, weight gain and neurodevelopment at 1 year.[13]

In another study, Bell et al, 2005 sought to determine whether a restrictive approach could reduce the number of PRTs without adverse complications in preterms. One hundred preterm neonates were randomized to either a restrictive or liberal PRT group. This study found that more IVHs and apneas occurred in the restrictive group, however further debates have occurred because initial cranial ultrasounds were not done.[11] Kirpilani et al 2006 performed a similar study termed PINT: Preterm Infant in Need of Transfusion study. The PINT study randomized 451 infants into a low and high threshold group. The authors concluded that in extremely low birth weight neonates, more PRTS occurred in the high threshold group without a corresponding increase in benefits. However, the Hb differences between the liberal and restrictive groups were small (1.1g/L).[12] In the Iowa trial, although with fewer subjects than the PINT trial, having only 100 participants, the Hb differences between the liberal and restrictive groups were larger (2.7g/L). This study found that a restrictive PRT guideline reduces the number of PRTs in preterm neonates and a liberal approach may be neuroprotective. In the Iowa trial, 6 cranial ultrasounds were found to be abnormal in the restrictive group compared to none in the liberal group, which included severe IVH's and periventricular leucomalacia. This result should be questioned because morbidity was not part of the initial aims of this study.[11] These studies concluded that short-term outcomes of restrictive versus liberal PRT practices showed no significant

differences in mortality or morbidity. Long-term outcomes did favour liberal transfusions because of better neurodevelopmental outcome at follow-up in some studies. [10-15] In recent years, many institutions have adopted a restrictive approach to reduce the amount of PRTs and donor exposures.

The CMJAH neonatal unit has 98 beds, including 14 Intensive Care Units, 8 Transitional Intensive Care Units, 70 Premature Units and 6 Kangaroo Mother Care beds. CMJAH is a tertiary, public sector hospital. Patients tend to originate from lower socio-economic backgrounds and mostly from areas within the city of Johannesburg. Currently we practice a restrictive approach to PRT in neonatal anaemia. See table 1 for CMJAH transfusion guidelines.

Despite numerous PRTs occurring in the neonatal unit, there is currently no data on neonatal PRTs in our unit. An audit has never been done on these transfusion guidelines and the decision to transfuse a neonate is at the discretion of the attending physician. Given the complications and cost of PRTs, it is essential firstly, that an audit on the existing records be done and further that a set of guidelines be established, to highlight problem areas for future research and to modify our practice where appropriate. As a starting point, this study aims to establish which neonates are receiving PRTs at CMJAH and to compare these neonates to those who did not PRTs.

Protocol

Aim

To review packed red cell transfusions in the neonatal unit at CMJAH

Objectives:

1. To determine demographic and clinical characteristics of neonates who received PRT and to compare them to neonates who did not receive a PRT.
2. To estimate the transfusion requirement of each neonate who received a PRT.
3. To establish the age at which the PRT was received.
4. To compare the incidence of IVH, ROP, BPD, NEC in neonates who received a PRT and those who did not.

Study design

This is a retrospective descriptive study of neonates who received PRT at CMJAH from 1 January 2013 to 31 December 2014. The units of PRTs issued per patient will be obtained from the blood bank. The age of the patient receiving PRT will be estimated from the date of the unit being issued.

Study population

Neonates admitted to CMJAH neonatal unit.

Inclusion Criteria

All neonates admitted within 72 hours of birth with records on the database for the study period.

Exclusion Criteria

Neonates with unobtainable information will be excluded.

Sample size prediction

Estimated study population would be about 3000 patients.

Variables

The demography such as gestational age, weight, apgars, gender would be recorded. The use of oxygen varying from nasal prongs to CPAP, IPPV or HFOV would be documented and the chronic use of oxygen. Number of transfusions would be recorded. Patient characteristics such as BPD(requiring supplemental oxygen for more than 28 days), ROP, significant NEC(grade 2 and above), IVH (cranial ultrasound), sepsis (culture proven) and death will be documented as well. Variables will be collected as per data sheet.

Limitations

This is a retrospective study, which has its limitations. The timing of the transfusion is unknown but can be estimated from the date the packed red cell was issued. The timing of the neonatal comorbidities is also unknown making it almost impossible to elude a causal relationship between blood transfusions and comorbidities.

Data analysis

Standard statistical methods will be used. Continuous variables with a normal distribution will be described using mean and standard deviations. Skewed data will be described using median and ranges. Categorical variables will described using frequencies and percentages. Comparisons between continuous variables will be done using independent T test or Mann Whitney test as appropriate. Categorical variables will be compared using the chi-square. Analysis will be done in birth weight categories using the stata pack.

Funding

Costs would be minimal due to the retrospective nature of the study.

Ethics

Application for ethics clearance will be submitted. We do not envisage any potential ethical issues. In addition, we would also apply for consent from the CEO of CMJAH and head of neonatal department.

References

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16. Ballot DE, Cooper PA, Davies VA, Chirwa P, Ramdin T. Neonatal Protocols CMJAH 2014. Updated 2014 Feb.

Table 1 :

The Charlotte Maxeke Academic Hospital transfusion guidelines:

1. The absolute indication for rapid blood transfusion is to restore tissue oxygenation and to expand blood volume after severe acute blood loss.
2. Shock secondary to blood loss.
3. Haemoblobin less than 13g/dL at birth
4. Haemoglobin less than 12g/dL in infants with severe lung disease (on ventilator), cyanotic heart disease or heart failure.
5. Haemoglobin less than 10g/dL if infant on supplemental oxygen but not ventilated
6. Haemoglobin less than 8g/dL if the infant has the following: unexplained tachycardia, recurrent apnoea, poor weight gain unexplained by other causes.
7. Haemoglobin less than 7g/dL if infant has no symptoms and reticulocyte count is $\leq 3\%$. If reticulocyte count is >3, do not transfuse and repeat Hb in 3-5 days. Transfuse if Hb continues to drop.

Neonatal transfusion questionnaire

Please encircle the appropriate item:

Blood transfusion

1. Was patient transfused during the stay?	Y	N		
2. How many transfusions occurred?	1	2	3 or >	
3. Inborn?	Y	N		

Mother

3. Booking bloods

RH	Positive	Negative	Unknown	
RPR	Positive	Negative	Unknown	
RVD	Positive	Negative	Unknown	

Demographic

4. Place of birth?					
5. Gender	M	F			
6. Gestation	<28w	28-30w	30-35w	35-37w	>37w
7. Birth weight?	ELBW	VLBW	LBW	NBW	
8. ICU stay	Y	N			
Length of ICU stay?	_____				
9. NPO2	Y	N			
If yes, on NPO2 at	28 days	36 weeks	d/c		
No. of days on oxygen?	_____				
10. CPAP	Y	N			
No. of days on CPAP?	_____				
11. IPPV/HFOV	Y	N			
No. of days on IPPV/HFOV?	_____				

Associated morbidities

12. Sepsis (blood culture proven sepsis)		Y	N
13. Pneumonia	(clinical)	Y	N
14. BPD	(Persistent O2 requirement >28/7 in terms and CA 36 weeks in prems)	Y	N

15. RDS		Y		N			
16. SVT		Y		N			
17. ROP		Y		N			
18. ROP GRADE	1		2		3		
19. IVH (seen on cranial u/s)		Y		N			
20. IVH GRADE	1		2		3		4
21. Apnoea		Y		N			

APPENDIX C: LETTER OF AMMENDMENT TO PROTOCOL

Dear Postgraduate MMed Panel

I have made the following amendments:

1. References numbered consecutively: All pages
2. Number of PRT's is 300000: 2nd paragraph of introduction
3. The reference of the above "number of PRT's is 300000" was incorrect and correct reference written in it's place: Reference no. 9 (previous no. 2)
4. The following references rewritten in Vancouver style: Reference 1, 2, 6, 9, 10, 11, 12, 13, 14, 15 now respectively reference 8, 9, 13, 1, 2, 3, 4, 5, 6, 7.
5. Grammatical errors corrected:
   Paragraph 1 line 3: has changed to have
   Paragraph 2 line 7/8: sentence rewritten
   Paragraph 6 sentence 1: sentence rewritten
   2nd last paragraph changed to 70 premature beds
6. Objectives 2 and 3 removed: Attempted getting the information from blood bank but the process was harder than anticipated.

APPENDIX D: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Xiaojun Song

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150976

NAME: (Principal Investigator)	Dr Xiaojun Song
DEPARTMENT:	Paeditrics Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A Review of Packed Red Cell Transfusions in the Neonatal Unit at Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	02/10/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof D Balott
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	28/10/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
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 resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX E: TURNITIN REPORT

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"Transfusions in the Preterm Infant", NeoReviews, 09/01/2007

A review of packed red cell transfusions in the neonatal unit at Charlotte Maxeke Johannesburg Academic hospital X Song, DE Ballot, T Ramdin Department of Paediatrics and Child Health, University of the Witwatersrand and Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg X Song, MBBCh, University of the Witwatersrand DE Ballot, MBBCh, FCPaed (SA), PhD, Neonatology (SA), University of the Witwatersrand T Ramdin, MBBCh, DCH, FCPaed(SA), Neonatology(SA), University of the Witwatersrand Authors: 1. X Song (jun_song_1109@yahoo.com) 2. DE Ballot (daynia.ballot@wits.ac.za) 3. T Ramdin (tanusharamdin@yahoo.com) The authors have no conflicts of interest to state. Abstract Background: Packed red cell transfusion (PRT) is a common therapeutic option in neonates with anaemia. Packed red cell transfusions are linked to certain neonatal complications such as Retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD), sepsis and more recently necrotizing enterocolitis (NEC). Even though many PRTs are given, the question of when to transfuse a neonate is still mostly based on opinion and differs from one institution to the next as well as between neonatologists. Despite numerous PRTS occurring in the neonatal unit, there is currently no data on neonatal PRTs in the CMJAH

neonatal unit Objectives: The aim of this study is to review PRT and it's associations. Methods: This is a retrospective descriptive study of all neonates younger than 72 hours old, who were admitted to the neonatal unit of Charlotte Maxeke Johannesburg Academic Hospital, from 1 January 2013- 31 December 2014. Neonates were divided into two groups depending on weight, smaller than 1500g and larger than 1500g. Within the groups, babies who were not transfused were compared with babies who were transfused. By means of univariate and multivariate analysis, the characteristics of the neonates and the incidence of complications were noted. Results: The study sample included 3432 babies. Of those, 2395 babies were bigger than 1500g and 1037 babies were smaller than 1500g. The total number of packed red cell transfusions (PRT) was 435/3432 neonates (12.67%) over a 2-year period. The incidence of NEC in the babies smaller than 1500g was 55.6% in the group that were transfused and 44.4% in the group that were not transfused (P<0.001). The incidence of NEC in the babies larger than 1500g was 48.3% in the group transfused and 51.3% in the group not transfused (P<0.001). The incidence of LOS in the babies smaller than 1500g was 66.4% in the group who were transfused and 33.6% in the group who were not transfused (P<0.001). However, the incidence of LOS was 36.3% in babies larger than 1500g who received transfusions and 63.7% in babies who did not receive transfusion (P<0.001). The incidence of BPD in the babies smaller than 1500g was 81.3% in the group transfused and 18.8% in the group that were not transfused (P<0.001). The incidence of BPD in babies bigger than 1500g was 63 .6% in the group transfused and 36.4% in the group not transfused (P<0. 001). The incidence of severe IVH in babies smaller than 1500g was 63.2% in the transfused group and 36.8% in the group not transfused (p<0. 001). The incidence of severe IVH in babies larger than 1500g was 58.3% in the transfused group and 41.7% in the not transfused group (p<0.001). The incidence of ROP in smaller than 1500g neonates was 70.2 % in the transfused group and 29.8 % in the not transfused group (p<0. 001). The lengths of stay as well as the duration of CMV in the transfused group were more than double the duration of those who were not transfused. In both groups, after multivariate regression analysis, a significant association was found between PRT and conventional mechanical ventilation (CMV), late onset sepsis (LOS), BPD, IVH and birth weight. NEC and ROP were no longer significantly associated with PRTs Conclusion: More transfusions were administered in babies smaller than 1500g compared to babies bigger than 1500g. Future prospective studies are need to determine whether PRTs cause LOS, BPD, IVH and longer duration of CMV and also to determine that NEC is not associated to PRT's. Background Packed red cell transfusion (PRT) is a common therapeutic option in neonates with anaemia. According to a Neonatal Transfusion International Forum held in 2009, very little is known about how many PRT's occur per neonate. 1 It was found at the forum that the birth weight of a neonate plays a substantial role in transfusions. 1 Strauss et al 1999, found that 90% of neonates below 1000g would receive a PRT. 2 He also estimated that 38000 premature neonates received more than 300000 PRTs annually. 2 According to a survey done by Lin and Hume 2007 the 5 large neonatal centres across Canada, which

included over 6000 neonatal admissions to NICU per year, and the PRT rates are about 7-23%. 1 The decision to transfuse a neonate is usually based on specific haemoglobin (Hb) and supplemental oxygen requirement. A number of randomized controlled studies have been done on different Hb levels to establish a safer and more evidence based Hb level at which to transfuse. 3,4 Studies about transfusing at a lower haemoglobin threshold to transfuse were considered restrictive, whereas liberal transfusion have a higher Hb threshold. Some studies found no difference between liberal transfusion and restrictive transfusion groups except for a higher number of PRTS in the liberal threshold group.3 Other studies found better neurodevelopmental outcome in the liberal transfusion groups.4 In recent years, many institutions, including Charlotte Maxeke Academic Hospital (CMJAH) neonatal unit, have adopted a restrictive approach to reduce the amount of PRTs and donor exposures. PRT's are linked to a number of neonatal complications such as retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD) and more recently necrotizing enterocolitis (NEC). 4,5,6,7 ROP may be associated with PRTs because anaemic neonates require longer supplemental oxygen and PRT requirements are also the highest in the extremely premature babies, who are also the mostly likely to develop ROP. Some studies have found no difference between IVH in a restrictive approach compared to the liberal transfusion groups. Two large randomized controlled trials were contradictory: The PINT (preterm infant in need of transfusion) study found that there was more PRTS in the higher threshold group without any increase in benefits. 5 The Iowa trial found that there were more severe IVH's in the restrictive group.4 BPD was associated with a larger volume of PRTs in some studies whereas other studies found no association between BPD and PRT. 6 The association between NEC and PRT may be explained by a reduction in the mesenteric blood flow after feeding is suddenly increased after a PRT. Many authors have done studies on the above associations but none are conclusive owing to the small size of the studies and because of many confounding factors. Newer studies show no association between NEC and PRT.7 Numerous PRTs occur in the CMJAH neonatal unit. However, the timing of transfusions is still opinion based and much research concerning the complications of PRTs is still needed. As a starting point, this study aims to establish which neonates are receiving PRTs at CMJAH neonatal unit and to compare them with the neonates who are not receiving PRT's by focusing on the number of neonates being transfused and the complications associated with this procedure. Methods This is a retrospective descriptive study of all neonates younger than 72 hours old, who were admitted to the neonatal unit of Charlotte Maxeke Academic Johannesburg from 1 January 2013- 31 December 2014. Neonates older than 72 hours, or with missing data, were excluded from the study. The data of the very low birth weight babies (VLBW) and bigger babies are heterogeneous and are therefore reported separately. The characteristics of neonates that received packed red cell transfusions (PRT) were noted. Neonates who received PRT were compared to neonates who didn't receive PRT and the clinical significance was calculated. The decision to transfuse packed red cells was based

on CMJAH neonatal unit transfusion guidelines as well as the opinions of the attending physicians. Neonates are transfused with a volume of 20ml/kg of PRT and given 1mg/kg of furosemide halfway through the transfusion. Database All neonatal records at CMJAH are captured on REDCap (Research Electronic Data Capture) when they are discharged. Data collected from REDCap neonatal database included the profile of the baby (inborn, age at admission, birth weight, gestational age and gender), as well as the maternal profile (RPR or HIV positive) and the duration of various modes of ventilation (CPAP, CMV and HFOV). Morbidities such as EOS, LOS, severe IVH, NEC, BPD, ROP, apneas, were also collected as well as the outcome (survival or death), duration of stay, and discharge weight. Definitions: RPR (rapid plasma reagin) is a screening test for syphilis HIV (Human Immunodeficiency Virus) CPAP (continuous positive airway pressure) CMV(conventional mechanical ventilation) HFOV (high frequency oscillatory ventilation) IVH (Intraventricular haemorrhage)- severe categorized as grade 2 or more BPD (bronchopulmonary dysplasia)- requiring oxygen at 28 days ROP (retinopathy of prematurity) NEC (necrotizing enterocolitis) - > grade 2 as per Bell's grading EOS (early onset sepsis) – culture proven sepsis occurring < 3 days old LOS (late onset sepsis) – culture proven sepsis occurring > 3 days old Statistical analysis Data from the neonatal database was initially uploaded into an MS Excel spreadsheet and edited. Variables not pertaining to the study were removed. The age of admission, duration of CPAP, duration of CMV and duration of HFOV, as well as the duration of the stay was calculated using formulas in the MS Excel spreadsheet. The data was then uploaded into the SPSS version 19, which is a statistical software package. Categorical variables were described using frequencies and percentages. Continuous variables, with a normal distribution, were described using mean and standard deviations. In order to test whether there was a significant difference between those who were transfused and those who were not transfused, the Chi squared test was used for categorical variables and the cut-off point for the P value was 0.001. An independent t test was used for comparing continuous variables. The data was subjected to a multivariate logistic regression analysis to derive the odds ratio and the 95% confidence interval to adjust for the differences. Ethics Professor P. Cleaton-Jones, chairperson of the human research ethics committee, granted ethics approval for the study. Clearance certificate no. M150976. Results A total of 3679 neonates were admitted to the Charlotte Maxeke Academic Johannesburg Hospital during the 2-year study period, of which 2395 babies were babies larger than 1500g and 1037 were babies smaller than 1500g. As per the exclusion criteria, 221 babies that were admitted after 72 hours were excluded. There were missing records for 16 babies and 10 babies were incorrectly classified (example <1500g babies appeared in the data of babies > 1500g) and were therefore also taken out of the study. This resulted in the eventual number of 3432 babies (larger and smaller than 1500g). The total number of packed red cell transfusions (PRT) was 435/3432 neonates (12.67%) over a 2-year period. Table 1: Comparison of the frequencies of the following characteristics between babies smaller and larger than 1500g Variable Babies <1500g N=1037 n=(%) Babies >1500g N=2395 n=(%) PRT 336

(32.4) 99 (4.1) Inborn 866 (83.4) 1811 (75.6) RVD exposed 300 (28.9) 695 (29) RPR exposed 13 (1.3) 32 (1.3) Female 552 (53.2) 970 (40.5) NCPAP only 617 (59.4) 333 (13.9) CMV 201 (19.4) 245 (10.2) HFOV 11 (1.1) 16 (0.7) EOS 36 (3.5) 63 (2.6) LOS 250 (24.1) 102 (4.3) O2 on day 28 208 (20) 33 (1.4) ROP 47 (4.5) Severe IVH 117 (11.3) 12 (0.5) NEC 72 (6.9) 29 (1.2) Survived 762 (73.4) 2276 (95) NEC surgery 19 (1.8) Table 2: Demographics of those transfused (T/F) and not T/F in babies smaller and larger than 1500g Demographic Babies >1500g Babies <1500g means T/F Not T/F T/F Not T/F Age on admission [days (SD)] 1.3 (0.64) 1.1 (0.42) 1.0 (0.2) 1.0 (0.2) Gestational age [weeks (SD)] 35.0 (3.74) 36.7 (3.44) 28.6 (2.8) 29.5 (2.1) Birth weight [grams (SD)] 2246.4 (642.83) 2646.0 (768.92) 1050.9 (256.8) 1153 (189.7) Duration of NCPAP [days (SD)] 3.9 (4.55) 2.5(2.12) 5.5 (7.7) 3.0 (2.5) Duration of ventilation [days (SD)] 14.4 (1.64) 4.6 (0.23) 11.9 (12.3) 4.3 (3.4) Duration of stay [days (SD)] 25.9 (2.44) 9.6(0.53) 46.4 (26.0) 22.8 (16.7) Table 3: Comparison of conditions in babies larger than 1500g, with and without transfusion. Condition(total) Transfused(%) Not transfused(%) P value Inborn (1811) 51(2.8) 1760 (97.2) <0.001 HIV exposed (695) 30(4.3) 665(95.7) 0.774 RPR exposed (32) 3 (9.4) 29 (90.6) 0.134 Male (1396) 62(4.4) 1361(97.5) 0.608 NCPAP (333) 25(7.5) 308 (92.5) 0.001 CMV (245) 60(24.5) 185 (75.5) <0.001 EOS (63) 7(11.1) 56 (88.9) 0.005 LOS (102) 37 (36.3) 65 (63.7) <0.001 BPD (33) 21(63.6) 12(36.4) <0.001 Severe IVH (12) 7(58.3) 5 (41.7) <0.001 NEC (29) 14(48.3) 15 (51.7) <0.001 Survived (2276) 79(3.5) 2197(96.5) <0.001 Table 4: Logistic regression in babies larger than 1500g with PRT as the dependent variable and GA, BW, BPD, EOS, LOS, BPD, Severe IVH as the independent variables. Conditions P value Inborn 0.025 CMV 0.000 LOS 0.000 BPD 0.000 Severe IVH 0.000 Outcome 0.006 Length of stay 0.018 Birth weight 0.000 Table 5: Comparison of conditions, in babies smaller than 1500g, with and without transfusion. Condition Transfusion(%) Not transfused(%) P value Inborn (866) 281(32.4) 585(67.6) 0.485 HIV exposed (300) 98(32.7) 202(67.3) 0.907 RPR exposed (13) 166(30.1) 386 (69.9) 0.470 Female (552) 166(30.1) 386(35.0) 0.209 NCPAP (617) 251(40.7) 366(59.3) <0.001 CMV (201) 137(68.2) 64 (31.8) <0.001 HFOV (11) 9(81.8) 2(18.2) <0.001 EOS (36) 9 (25.0) 27(32.7) 0.522 LOS (250) 166(66.4) 84(33.6) <0.001 BPD (208) 169(81.3) 39(18.8) <0.001 ROP (47) 33(70.2) 14(29.8) <0.001 Severe IVH (117) 74(63.2) 43(36.8) <0.001 NEC (72) 40 (55.6) 32(44.4) <0.001 NEC surgery (19) 14(73.7) 5(26.3) <0.001 Survived (762) 256(33.6) 507(66.5) 0.323 Table 6: Logistic regression in babies smaller than 1500g with PRT as the dependent variable and GA, BW, BPD, EOS, LOS, BPD and Severe IVH as the independent variables. Conditions P value CMV 0.000 LOS 0.000 BPD 0.000 Severe IVH 0.001 Survived 0.006 Length of stay 0.023 Birth weight 0.000 Discussion This was the first study at the CMJAH neonatal unit to look at packed red cell transfusions. Previously Niermeyer and Velaphi studied the delay of cord clamping aimed at improving the hemodynamic status after birth to increase red blood cell mass and haemoglobin in order to reduce the need to transfuse.8 During the 2-year study period, approximately a-tenth (12.4%) of the 3432 neonates in the study received PRT. The 12.4% is similar to a survey done by Lin and Hume who found that PRT rates were about 7-23%. 1

Strauss et al also estimated that 38000 premature neonates received more than 300000 PRT's annually, which is 12.67%, and which is very similar to CMJAH's transfusion rate of 12.4%.2 In this study, more neonates smaller than 1500g had a PRT (32.4%) compared to babies larger than 1500g (4.1%). The incidence of NEC in the babies smaller than 1500g was 55.6% in the group that were transfused and 44.4% in the group that were not transfused (P<0.001). The incidence of NEC in the babies larger than 1500g was 48.3% in the group transfused and 51.3% in the group not transfused (P<0.001). The results in the babies larger than 1500g were possibly biased because of a smaller group of babies having NEC, 29 babies compared to 75 in the smaller than 1500g group. The association between NEC and PRT was no longer significant after an adjustment for confounding variables in a logistic regression analysis. In recent retrospective studies by Wallenstein et al Oct 2014 and Sharma et al Sept 2014 they found no association between PRT and NEC similar to our findings.9,10 Contrary to previous studies, a retrospective case-control study by AlFaleh et al May 2014 found that neonates receiving PRT's have a lower incidence of NEC.11 A recent prospective study by Patel et al Mar 2016 found that babies smaller than1500g with severe anaemia, and who did not receive PRC, had an increased risk of NEC.7 The incidence of LOS in the babies smaller than 1500g was 66.4% in the group who were transfused and 33.6% in the group who were not transfused (P<0.001). The higher incidence of LOS in the babies smaller than 1500g receiving transfusion could be explained by the fact that ill and smaller babies are more likely to be transfused. However, the association of LOS and PRT could be explained by a retrospective study by Jufferman et al who looked at the association between the development of nosocomial bacterial infection and transfusion of leukodepleted PRTs.12 They concluded that PRTs are associated with the onset of secondary bacterial infection in patients with sepsis possibly due to immunomodulatory effects of PRTs contributing to the adverse outcome in the recovery phase of sepsis.12 However, the incidence of LOS was 36.3% in babies larger than 1500g who received transfusions and 63.7% in babies who did not receive transfusion (P<0.001). This shows that there is less likelihood of an association between PRTs and LOS. The incidence of BPD in the smaller than 1500g babies was 81.3% in the group transfused and 18.8% in the group that were not transfused (P<0.001). The incidence of BPD in bigger than 1500g was 63 .6% in the group transfused and 36.4% in the group not transfused (P<0. 001). The association between BPD and PRT persisted only in the VLBW group after an adjustment was made to the confounding factors in a logistic regression analysis possibly due to birth weight being a significant confounding factor. A retrospective study by Zhang et al Sept 2013, performed on neonates with a birth weight smaller than 1500g or a gestational age less than 32 weeks found that there was an association between BPD and PRT even after the adjustment of the confounding variables including the birth weight, gestational age, surfactant use and the duration of ventilation.6 Because the temporal relationship is unknown, we are unable to say whether blood transfusions caused BPD or that babies with BPD are more likely to receive a transfusion. In our unit, babies with BPD and low Hb are transfused to facilitate their being weaned off oxygen.

The incidence of severe IVH in the babies smaller than 1500g was 63.2% in the transfused group and 36.8% in the group not transfused (p<0. 001). The incidence of severe IVH in the babies larger than 1500g was 58.3% in the transfused group and 41.7% in the not transfused group (p<0. 001). After the adjustment was made with logistic regression analysis, the association between severe IVH and PRT persisted only in the group of babies smaller than 1500g. A study by Christensen et al Jan 2014 found there was an association between PRT in the first days of life and IVH.13 However, Christensen was unable to say that early PRT caused the IVH, however, a reduction in PRTs is associated with a decrease in the incidence of severe IVH. Though there is an association, a significant bleed will result in a drop in Hb and thus a subsequent blood transfusion is always necessary in these cases. The incidence of ROP in smaller than 1500g neonates was 70.2 % in the transfused group and 29.8 % in the not transfused group (p<0. 001). ROP's association with PRT was no longer significant after the logistic regression analysis. The incidences of ROP in bigger babies were not recorded because it is a disease related to prematurity. A prospective study by Mohaghegi et al 2003, showed no significant difference in ROP between the babies that were transfused liberally and those that were not.14 This is similar to the findings in this study about ROP not being significantly associated with blood transfusion. The lengths of stay as well as the duration of CMV in the transfused group were more than double the duration of those who were not transfused. The increased duration of both, hospital stay and ventilation, in babies who were transfused is possibly because those babies who received transfusions are likely to have been more ill and would, therefore, require longer medical care. There are currently very few studies available on the association between PRT's and CMV. Conclusion Approximately 12.4% of neonates admitted to CMJAH neonatal unit receive a PRT. Neonates, smaller than 1500g, are 8 times more likely to be transfused than babies bigger than 1500g. Two large study groups that were compared depending on weight less than 1500g and larger than 1500g. Within the groups, babies who were transfused were compared to babies who were not transfused and the incidence of complications were noted. In both groups, after multivariate analysis, a significant association found between PRT and CMV, LOS, BPD, IVH and BW. NEC and ROP were no longer significant. This is in keeping with a study by Patel et al in 2016, which stated that PRC was not associated with NEC but with severe anaemia. A study by Mohaghegi et al 2003 also found that ROP was not associated with PRT. The results for LOS were contradictory. The incidence of LOS was higher in the transfused group of babies smaller than 1500g and equal in the group of babies larger than 1500g. Therefore, further research should be conducted to establish if the association is significant. BPD is strongly associated with PRT and is backed by a retrospective study by Zhang et al. Further prospective studies will be required to find a causal relationship. IVH is also strongly associated with PRT but severe IVH will result in a bleed requiring PRT and because a temporal relationship is unknown, we are uncertain whether IVH caused PRT or vice versa. Smaller and more ill babies are also more likely to receive transfusion; however this bias was decreased with a multivariate analysis. This study should serve as a good starting point for further studies as

well as for changing the collection requirement of the neonatal database to help with further studies such as the date of transfusion, number of transfusions, Hb at which transfused and to state the date of complications. This study also shows that PRT is not benign and can be associated with multiple complications and therefore physicians should keep in mind the possible complications when giving babies transfusions. References 1. New HV, Stanworth SJ, Engelfriet CP, Reesink HW, McQuilten ZK, Savoia HF, et al. Neonatal transfusions. Vox sanguinis. 2009;96 (1):62-85. 2. Strauss RG. Data-driven blood banking practices for neonatal transfusions. Transfusion. 2000;40:1528-1540 3. Bifano E. The effect of hematocrit (HCT) level on clinical outcomes in extremely low birthweight (ELBW) infants. PediatrRes. 2001;49:311A. 4. Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics. 2005;115(6):1685-91. 5. Kirpalani H, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, et al. The Premature Infants in Need of Transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. The Journal of pediatrics. 2006;149(3):301-7. 6. Zhang Z, Huang X, Lu H. Association between red blood cell transfusion and bronchopulmonary dysplasia in preterm infants. Sci Rep. 2014;4:4340. 7. Patel RM, Knezevic A, Shenvi N, Hinkes M, Keene S, Roback JD, et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very low-birth-weight infants. Jama. 2016;315(9):889-897. 8. Niermeyer S, Velaphi S. Promoting physiologic transition at birth: re-examining resuscitation and the timing of cord clamping. Seminars in fetal & neonatal medicine. 2013;18(6):385-92. 9. Wallenstein MB, Arain YH, Birnie KL, Andrews J, Palma JP, Benitz WE, et al. Red blood cell transfusion is not associated with necrotizing enterocolitis: a review of consecutive transfusions in a tertiary neonatal intensive care unit. The Journal of pediatrics. 2014;165(4):678-82. 10. Sharma R, Kraemer DF, Torrazza RM, Mai V, Neu j, Shuster JJ et al. Packed red blood cell transfusion is not associated with increased risk of necrotizing enterocolitis in premature infants. J Perinatol. 2014; 34(11):858-862. 11. AlFaleh K, Al-Jebreen A, Baqays A, Al-Hallali A, Bedaiwi K, Al-Balahi N, et al. Association of packed red blood cell transfusion and necrotizing enterocolitis in very low birth weight infants. Journal of neonatal-perinatal medicine. 2014;7(3):193-8. 12. Juffermans NP, Prins DJ, Vlaar AP, Nieuwland R, Binnekade JM. Transfusion- related risk of secondary bacterial infections in sepsis patients: a retrospective cohort study. Shock. 2011;35 (4):355-9. 13. Christensen RD, Baer VL, Lambert DK, Ilstrup SJ, Eggert LD, Henry E. Association, among very-low-birthweight neonates, between red blood cell transfusions in the week after birth and severe intraventricular hemorrhage. Transfusion. 2014;54(1):104-8. 14. Mohaghegi P, Khosravi N, Samaii H. Retinopathy of Prematurity and Blood Transfusion Protocols. Iranian Journal of Public Health. 2003;32(4):64-67 1 2 3 4 5 6 7 8 9 10 11 12 13 14