Whole blood versus red cell concentrates for children with severe anaemia: a secondary analysis of the Transfusion and Treatment of African Children (TRACT) trial

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Running Title: Whole Blood versus cell concentrates for Transfusion in African Children

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Research in Context

Evidence before the study

In sub-Saharan Africa (sSA), blood transfusion services are moving towards a wider provision of red cell concentrates for transfusion rather than using whole blood, reflecting practice in high-income countries. These changes have largely been justified on the grounds of maximizing the utility of a single donation of blood, despite red cell concentrates being more costly and time-consuming to prepare than whole blood. A published systematic review on transfusion practice in sSA in 2019, reviewed recommendations and evidence supporting guidelines regarding whole blood and packed red cell transfusions for pregnancy-related indications and paediatric anaemia. From 15 sSA countries, 32 English language guidelines were identified. Seven justified use of red cell concentrates rather than whole blood, largely on the basis of safety (whole blood was considered to increase the risk of volume-related complications). None cited evidence to support any of their recommendations. The international Transfusion and Anemia Expertise Initiative (TAXI) developed consensus recommendations on red blood cell transfusion in critically ill children in 2018. They noted that one major challenge for developing and justifying their recommendations was the paucity of published paediatric data. They made no specific recommendations on use of whole blood versus red cell concentrates. Prior to the Transfusion and Treatment of severe Anaemia in African Children (TRACT) trial there was scant evidence to support the introduction of red cell concentrates for paediatric transfusion in Africa. In addition, there have been no clinical trials comparing whole blood and packed red cell transfusion on patient outcomes.
The multi-centre factorial TRACT trial, designed to establish evidence-based transfusion practice, investigated whether immediate transfusion in children with uncomplicated versus no transfusion (standard of care) in children with uncomplicated severe anaemia (haemoglobin 4-6g/dl) and whether a higher volume of transfusion 30ml/kg whole blood (or red cell concentrate equivalent) in all children with Hb < 6 g/dl would improve outcomes compared to the standard volume of 20ml/kg whole blood (or red cell concentrate equivalent) defined as mortality to day 28 (primary outcome) and to day 180, readmissions and the relapse of severe anaemia. The trial demonstrated that not immediately transfusing uncomplicated severe anaemia was safe; provided children were monitored (for development of severity) requiring transfusion. The trial also demonstrated higher volumes of blood transfusions (30mls/kg whole blood equivalent) halved the number of deaths, in children without fever. Whereas children admitted to hospital with a fever (axillary temperature >37.5C) 20mls/kg whole blood equivalent were about half the risk of dying compared to those who received a higher volume. Transfusion with whole blood and red cell concentrates were used in the trial but did not affect the primary or safety endpoints.

Added value of this research

In TRACT trial factorial randomisation examining optimum volume of blood for transfusion involved 3992 transfusions which included 1632 (41%) whole blood and 2360 (49%) red cell concentrate transfusions (issued by the blood bank but were not prespecified on the request form). A further analysis of haemoglobin recovery by pack type (whole blood versus red cell concentrates) at 8 hours showed superior recovery in children initially receiving whole blood ($p<0.0001$). Children whose initial transfuse with red cell concentrates had more second transfusion episodes and longer hospital stays compared to children who received whole blood initially. Pack-type, supplied for the initial transfusion, did not predict any other clinical outcomes including mortality at 28 or 180 days, or hospital readmissions. Transfusion-related adverse events related to volume overload were rare and unrelated to pack type. Although not a randomised comparison, observational analyses of trial

data indicate that children who received red cell concentrates as their first transfusion had poorer haematological correction, more additional transfusions (which increases infection risk through more donor exposure and further depletes scarce supplies of blood) and longer hospital stays without any demonstrable safety benefits.

Implications of all the available evidence

Providing whole blood for paediatric transfusion, which accounts for a large proportion of transfusions in sSA, could result in substantial cost and resource savings for blood transfusion and health services.

Background

The multicentre Transfusion and Treatment of African Children (TRACT) trial established best evidence on the timing of transfusion in children with uncomplicated anaemia (haemoglobin 4-6g/dl) and optimal volume (20 versus 30ml/kg whole blood (or 10 vs 15ml/kg red cell concentrates) for transfusion in children hospitalised with severe anaemia (Hb <6g/dl) on Day 28 mortality (primary endpoint) and secondary endpoints including safety. As evidence on the safety of blood components is limited we undertook a secondary analysis comparing children receiving whole blood versus red cell concentrates as their initial transfusion on clinical outcomes.

Methods

This analysis includes 3188 children with severe anaemia (Hb <6g/dl) who received either whole blood or red cell concentrates. Whole blood or cell concentrates were issued routinely by the blood transfusion services, but not prespecified on the request form. The impact of blood pack type on haematological correction, re-transfusion, and other clinical endpoints was explored using multivariate regression models.

Findings

1632/3992 (41%) transfusions in 3188 children were whole blood. Compared with whole blood, children receiving cell concentrates in their first transfusion had less haemoglobin recovery at 8 hours (packed cells mean(95%CI): -1.3(-1.5,-1.0) 20ml/kg arm,-1.4(-1.6,-1.1) 30ml/kg; settled cells mean(95%CI) -1.1g/dl(-1.2,-0.9) 20ml/kg arm, -1.5g/dl(-1.7,-1.3) 30ml/kg arm; p<0.001 for pack type comparisons, p=0.003 heterogeneity by arm), higher odds of receiving a second transfusion [ORs 2.32 (95%CI 1.30,4.12) and 2.97 (2.18,4.05) respectively; p<0.001], and had a longer time to discharge [sub-Hazard Ratios 0.94 (95%CI 0.81,1.10) and 0.86 (95% CI 0.79,0.94) respectively; p=0.002]. No child developed features of cardio-pulmonary overload.

Interpretation

Whole blood is safe to use in children, resulting in superior haematological correction, less repeat transfusion and shorter hospital stays. These findings have substantial cost implications for both blood transfusion and health services. Nevertheless, a clinical trial comparing whole blood transfusion to red cell concentrates maybe needed to convince policy makers.

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Introduction

Most transfusions given in sub-Saharan Africa (sSA) are with whole blood[1]. Reflecting international practice, blood transfusion services (BTS) across sSA are increasingly moving towards a wider provision of red cell concentrates, despite clear differences in the patient populations requiring transfusion[2]. Although more costly and time-consuming to prepare, these changes have largely been justified on the grounds of maximizing the utility of a single donation of blood (https://www.who.int/bloodsafety/processing/en/). Data on requirements for blood components are scanty for most African countries. Studies published on this subject tend to be biased towards blood transfusion services (BTS)[3,4], and may not represent the needs of less well-resourced hospital blood banks. In general, requirements differ from those in many richer countries, where oncology and surgery are the biggest users of transfusion components; whereas in sSA, demand predominantly centres on acute paediatric severe anaemia (largely due to nutritional and infectious (malaria, sepsis and helminths) causes and sickle cell disease) , and the haemorrhagic complications of pregnancy and trauma[5]. Transfusions required by these patient groups are largely for emergency management, where the deficit to be replaced is whole blood. The evidence supporting the superiority of red cell components (packed cells) over whole blood in children and pregnant mothers, the most common recipients in much of sSA, is weak[6]. There is some evidence that it is safe to use whole blood in African children hospitalised with severe anaemia, and that this does not lead to fluid overload events[7], but most studies have been too small to provide reliable information for guideline recommendations. With respect to international guidelines for paediatric transfusion in critically ill children, the international Transfusion and Anemia Expertise Initiative (TAXI) developed consensus recommendations for red cell transfusion in 2018[8]. The guideline group noted the general paucity of published paediatric data to justify their recommendations. Moreover, there were no specific recommendations on the use of whole blood versus red cell concentrates.

The multi-centre open-label Transfusion and Treatment of severe Anaemia in African Children (TRACT) trial with a factoral design was conducted to establish evidence-based transfusion and treatment strategies with the aim of improving both early and long-term mortality and readmission[9]. The trial was registered as ISRCTN84086586 (date 11/02/2013). One transfusion randomisation (n=3196) compared a higher volume of transfusion 30ml/kg whole blood (or 15mls/kg red cell concentrate equivalent) to the standard volume of 20ml/kg whole blood (or 10mls/kg red cell concentrate) on mortality to day 28 (primary outcome) and day 180, readmissions and safety (See Supplemental Figure (SF)1: Trial consort). By Day 28 receipt of 30 mls/kg whole blood equivalent,

compared to 20 mls/kg in children without fever halved the number of deaths [hazard ratio (HR) 0. 43, 95% confidence interval (CI) 0.27–0.69]. Whereas giving children who had a fever (>37.5°C) 30mls/kg volume almost doubled the risk of mortality at 28 days in comparison to those who received 20mls/kg volume [HR 1.91, 95%CI 1.04-3.49; p-value for heterogeneity p=0.00009] [10]. One or more serious adverse events (SAE), largely readmissions, occurred in 431(27%) higher volume vs. 416(26%) lower volume group participants; allergic reactions occurred in 25(2%) 30ml/kg vs. 20(1%) 20ml/kg children (p=0.55; no deaths), suspected pulmonary/cardiovascular SAE in 2(<1%) vs. 3(<1%) (2 deaths), respectively (p=1.00) and neurological (grade 3) SAE in one (30ml/kg) (see Supplemental Table (ST)1). The other transfusion analysis (n=1565) comparing immediate transfusion to no immediate transfusion (control) was reported separately[11].

Both whole blood and red cell concentrates were used within the trial. Given the paucity of data on the relative merits of the different pack types on clinical outcomes in the management of children admitted to hospital with severe anaemia, the major objective of this analysis was to examine the relative safety and effectiveness of different pack types (whole blood versus red cell concentrates) on haematological correction, requirement for re-transfusion and the main trial clinical endpoints.

Materials and Methods

Study design and participants

This is a secondary analysis of the TRACT trial data restricted to those who received an immediate transfusion[10]. The trial was an open-label, multicenter, factorial randomized trial in three hospitals in Uganda and one in Malawi, enrolling children aged 2 months to 12 years admitted with severe anaemia (haemoglobin<6g/dl) (see Trial Consort Supplemental (S)Figure 1 including inclusion and exclusion criteria). When prior written consent from parents or legal guardians could not be obtained, ethics committees approved verbal assent with delayed written informed consent as soon as practicable.[12] Otherwise, informed written consent was obtained from parents or guardians. The ethics committees of Imperial College London, UK (ICREC_13-1-11), Makerere University, Uganda (SOMREC 2013-050), and College of Medicine, Blantyre, Malawi (COMREC P.03/13/1365) approved the protocol.

Procedures

The details of management and trial conduct have been published previously[10]. Briefly, a structured clinical case report form and baseline investigations was completed at admission. Bedside

observations were performed at admission and every 30 minutes for the first 2 hours, then 4, 8, 16, 24, and 48 hours after the start of the first transfusion. Haemoglobin was assessed 8-hourly in the first 24 hours, then at 48 hours, or if triggered by clinical deterioration, using Hemocue®[13]. Patients were actively monitored for serious adverse events (SAEs), particularly suspected cardiac or pulmonary overload or transfusion-related events following modified guidelines recommended by the United Kingdom's Serious Hazards of Transfusion initiative.[14] Post-discharge, children were clinically assessed and haemoglobin measured at 28, 90 and 180 days.

In the trial all blood products were supplied free of charge to patients and hospitals. Red cell concentrates were either gravity-prepared (settled cells) or prepared by centrifugation (packed cells)[15]. The pack-type (supplied by the blood banks) used was based on availability at the time and was without reference to clinician preference or patient need, nor was it directed by the protocol of the trial (Supplemental methods for details).

Whole-blood and packed/settled cells were transfused over 3-4 hours or 2-3 hours respectively. Additional transfusions were permitted for new or persistent haemoglobin levels of <4g/dl or severity features (see above) if haemoglobin remained <6g/dl. At each clinical assessment clinicians examined children for denovo features of severity, drops of haemoglobin (requiring additional transfusion) and actively solicited suspected transfusion-related adverse events (febrile reactions, TRALI (Transfusion Related Acute Lung Injury) and TACO (Transfusion Associated Cardiac Overload)[9].

Outcomes

The outcomes for this analysis were haemoglobin recovery at 8 hours and 180 days, requirement for re-transfusion, length of stay, changes in heart and respiratory rates to Day 180, and the main trial clinical endpoints (mortality to Day 28, and Day 180, and readmissions to Day 180).

Statistical analysis

Baseline characteristics of children and characteristics of packs used for transfusions were summarised and children's haemoglobin levels (mean and 95% CI) over time were plotted by characteristics of the first pack of the first transfusion. The impact of blood pack type (the first pack of the first transfusion) on different clinical outcomes was explored using multivariate regression models. These were either previously published models (for mortality and readmissions, detailed in the supplementary material)[10,16] or were built using backwards elimination with a threshold of

p<0.1, with candidate covariates to enable adjustment for confounders identified through clinical input/prior knowledge and review of the literature and checking for non-linearity of continuous variables using fractional polynomials with Stata's mfp function (alpha=0.05). All models were adjusted for site, if not already retained. Cox regression was used for mortality models and Schoenfeld residuals were tested and checked graphically for non-proportionality. Receipt of a second transfusion in the initial admission was investigated using logistic regression. Haemoglobin recovery at 8 hours and 180 days was examined with normal linear regression, adjusted for baseline haemoglobin. Time to discharge was modelled using competing risks with the competing event as death during initial admission and a sub-hazard ratio <1 indicating longer time to discharge; for time to readmission models, the competing event was death post-discharge. The competing risk methods estimated the probability of the event (analogous to Kaplan-Meier) using cumulative incidence functions and estimated the effect of each variable adjusted for other variables on the subdistribution hazard corresponding to the cumulative incidence function[17]. Blood pack type was then added into these models if not already retained, to examine if it was independently predictive of the outcome. Possible interactions in outcome models for blood pack type and randomisation arm were considered prior to analyses and tested using a likelihood ratio test or Wald test and checked graphically, p-values from the 10 models were compared to Benjamini-Hochberg critical values to adjust for multiple testing.[18] Models used complete case analysis as missing data was <5% for baseline values. Absolute numbers and outcome events by children receiving the different blood pack types were also summarised with unadjusted estimates of effect estimated (Table S2). Respiratory rate and heart rate from randomisation to 48 hours post transfusion start were modelled using change from baseline as the outcome in a normal GEE model, adjusting for baseline value (detail in supplementary material)[19] Sensitivity analyses were carried out restricting data to Soroti and Mbale, Uganda for comparisons of whole blood vs settled cells, and Mulago, Uganda and Blantyre, Malawi for comparisons of whole blood vs packed cells (given distribution of packs across sites in Table 1). Analyses were also carried out in malaria and HIV subgroups. Results were similar to analyses on all children (Tables S13,S14). Stata v16.1 was used for analyses.

Role of funding Source

The funder of the study had no role in trial design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had the final responsibility to submit for publication.

Results

Between 17 September 2014 and 15 May 2017, 3199 children with a haemoglobin <6g/dl were enrolled into the trial. Consent was declined for three participants post-admission, leaving 3196 for further analysis. 2418 (76%) of these participants had a haemoglobin level of <4g/dl and/or severity features, 778 had uncomplicated severe anaemia. 7 children died prior to transfusion and 1 had no form confirming transfusion leaving 3188 children considered in the current analysis.

The baseline characteristics of these participants are described in Table 1, stratified by the first pack type used in their initial transfusion. The median age was 37 months (IQR 18-64). 2045 (64%) had malaria, 589 (18%) had haemoglobinuria and 1338 (42%) had a haemoglobin of <4g/dl at screening. Although statistically significant due to the size of the trial, differences in baseline characteristics between pack types were modest in size. Soroti and Mbale, Uganda, predominantly used whole blood and settled cells, Malawi, Blantyre, predominantly whole blood and packed cells, and Mulago, Uganda all three pack types.

There were 3992 transfusions started in 3188 children during their primary admission. 2692 (84%) children received one transfusion, 349 (11%) had two transfusions and 147 (5%) received three or more transfusions during their initial admission. Whole blood was the first pack provided for 1632/3992 (41%) transfusions, of which 1101/1632 (67%) were adult packs and 531/1632 (33%) were from transfer packs. Of red cells concentrates, 844/2360 (36%) were packed cells while 1516/2360 (64%) were settled cells. Adherence to volumes directed by the protocol (20ml/kg or 30ml/kg for whole blood and 10ml/kg or 15ml/kg for packed or settled cells) for initial transfusions were within ±1.5ml of expected volumes in 681/692 (98%) for packed cells and 1029/1092 (94%) for settled cells, and were within ±3ml of expected volumes in 1367/1404 (97%) for whole blood transfusions.

486 children required two or more packs in their initial transfusion. Only 109/486 (22%) had two or more different types of pack and 1 had missing information on their second pack type. In 78/109 children there were different types of whole blood pack (i.e. transfer instead of direct or vice versa), and 4/109 children had different types of red cell concentrate (i.e. packed cell instead of settled cell packs or vice versa). Only 27 children (0.8% of those transfused or 6% of those receiving two or more packs in their first transfusion) received both whole and packed/settled cells in their first transfusion.

The whole blood packs had a median (IQR) storage length ('age') of 10 (5-18) days, compared to 12 (7-20) days for packed cells and 13 (7-19) days for settled cells. As would be expected, the

haemoglobin levels in packed cells and settled cells were higher (median 19.5g/dl and 16.9g/dl respectively) than those in whole blood (13.9g/dl) as were haematocrits (median 60.0% and 52.1% respectively, compared to 42.1% in whole blood) (Table 2).

Children's haemoglobin recovery was greatest in the first 8 hours following randomisation and stabilised before increasing further between 48 hours and 28 days (Figure 1). Haemoglobin recovery by 8 hours was lower in those who received packed cells or settled cells ($p<0.0001$) compared to whole blood. The difference was mean (95%CI) -1.4g/dl (-1.6,-1.1) in 30ml/kg and -1.3g/dl (-1.5,-1.0) in 20ml/kg with packed cells, and -1.5g/dl (-1.7,-1.3) in 30ml/kg and -1.0g/dl (-1.2,-0.9) in 20ml/kg with settled cells ($p<0.0001$ overall; and $p=0.003$ for heterogeneity between volumes) (Table 3, Table S10). Haemoglobin recovery was slightly lower following transfusion with older blood and blood packs with lower haemoglobin levels (Figure 1). There was no evidence of differences in haemoglobin recovery at 180 days for children receiving packed or settled cells and those receiving whole blood (Table 3, Table S11).

Compared to whole blood, children who received blood as packed or settled cells in their first transfusion had higher odds of receiving a second transfusion (2.32 95% CI (1.30,4.12) and 2.97 (95% CI 2.18,4.05) respectively; $p<0.001$ overall) and had longer hospital stays (sHR 0.94 (95% CI 0.81,1.10) and 0.86 (95%CI 0.79,0.94); $p=0.002$) (Table 3,Tables S9,S12), Figure 1e). There was no evidence of association between type of blood supplied for the first transfusion and other clinical outcomes, including mortality at 28 or 180 days, or readmissions for any cause (Table 3, Tables S3-S8, Figure S1a-d).

Most children presented with severe tachycardia and tachypnoea (mean 146 bpm and 44 brpm respectively), but this resolved over time in both those receiving whole blood and packed or settled cells as their first transfusion (Figure 2, Table S15). Heart rates decreased more quickly in those receiving whole blood (global $p=0.0001$), and respiratory rates decreased more quickly in those receiving either whole blood or settled cells (Figure 2, Table S15) (global $p=0.001$). Differences for heart rate at 48 hours were mean (95%CI) 4.6bpm (0.3,8.8) in 20ml/kg and 3.1bpm (1.1,5.0) in 30ml/kg comparing whole blood to packed cells and 4.0 (0.1,7.9) beats per min (bpm) in 20ml/kg and 4.8bpm (3.1,6.5) in 30ml/kg comparing whole blood to settled cells. Differences for respiratory rate at 48 hours were mean (95%CI) 1.5 (-0.4,3.4) breath per minute (brpm) in 20ml/kg and 0.9 (-0.02,1.7) for 30ml/kg comparing whole blood to packed cells and 0.1 (-1.6,1.8) brpm in 20ml/kg and 0.01 (-0.7,0.7) in 30ml/kg comparing whole blood to settled cells.

Discussion

The multi-centre TRACT trial provided the opportunity to investigate whether an initial transfusion with whole blood or either packed or settled cells (red cell concentrates) influenced clinical outcomes in children admitted to hospital with severe anaemia. We found that haematological correction at 8 hours was substantially better in children who received whole blood than in those transfused with either packed or settled cells, whether or not they received a higher volume of blood. Children receiving red cell concentrates as their initial transfusion therefore had a higher number of additional transfusions and longer hospital stays. There was no evidence that type of blood was associated with mortality or readmissions.

These findings have important implications for blood transfusion services in Africa that are promoting the use of blood components, particularly given the additional staff and equipment requirements and consequently overall costs. The Blood Transfusion Safety Programme for the World Health Organization recommends that blood transfusions collected may be used more effectively if they are separated into components (red cell concentrates, fresh frozen plasma, cryoprecipitates and platelet concentrates), so that they can be utilised by more than one patient. Moreover, the Global Database on Blood Safety uses the proportion of components prepared per unit of whole blood collected as a general indicator of the productivity of blood transfusion services. The 2016 report indicated that only 36% (15/41) of African countries contributing data to the audit had <25% of whole blood donations separated into components[1]. Our findings indicate that achieving exclusive component preparation will have a negative impact on paediatric services, one largest users of transfused blood[2,20].

Potential volume overload is another reason why red cell concentrates have been recommended[5]. In our study, 42% of children had Hb<4 g/dl, and 27% had sickle cell disease (SCD). These groups may be at risk of volume overload and/or transfusion reactions, since children with profound anaemia are considered at higher risk of heart failure and many children with SCD have had multiple previous transfusions[11,21]. However, we found no evidence of overload in children receiving whole blood compared to red cell concentrates, or with a larger volume of blood received, which was irrespective of severity of anaemia or underlying SCD[10,22]. Only 5 episodes of suspected possible transfusion related lung injury or cardiac overload were reported in the trial. Four of these episodes occurred in children receiving packed cells and one in a child receiving whole blood. Most were adjudicated by the endpoint review committee (who were unaware of the treatment assignments)

to be unrelated to the intervention[10]. In the TRACT trial, no child received a diuretic or anti-failure medication, and although there were some differences between pack types in heart rate and respiratory rate at 48 hours (Figure 2), these were too small to be clinically meaningful.

The major limitation of the study is that the trial data used for the sub-analysis did not directly compare the use of whole blood versus packed or settled cells through a specific randomisation. However, the large size of the trial, in which donor blood was sourced from 3 regional blood transfusion services in two countries where blood was issued on the basis of availability rather than prespecified by request or clinician preference, provides some assurance against potential bias. Our models were also adjusted for site and other risk factors for each outcome. Moreover, the recording of the donor blood haematological characteristics was largely done by dedicated laboratory staff unaware of the pack type.

One of the most puzzling findings is that haematological correction was worse in the children receiving packed or settled cells than in those receiving whole blood. Indeed, the opposite would be expected given that whole blood is likely to result in more haemodilution in the recipient than packed/settled cells. One obvious explanation is that the haemoglobin concentration of the packed or settled cell packs was lower than the required range. However, our quality-control measures demonstrated that the median and interquartile ranges for haemoglobin concentration in packed cells, settled cells and whole blood were all within the expected range, making this unlikely[15]. We also weighed each pack and recorded the volume of blood transfused, allowing us to exclude non-adherence to transfusion volume randomisation. We conclude, therefore, that guidelines stating that 10ml/kg of red cell equivalent (as either packed or settled cells) equivalent to 20ml/kg of whole blood are not correct and should be reviewed. However, given the added expense of preparing components, revisions to the guideline to increase the volumes of red cell components to the whole blood equivalent would involve a more complex calculation: our data suggest that 30ml/kg whole blood equates to approximately 20ml/kg packed/settled cells. Moreover, we have shown that for children with severe anaemia, using red cell concentrates does not yield additional benefits in terms of safety or efficacy. It does however, at the current recommended volumes, expose children to additional transfusions, thus more ‘donor exposure’ with the inherent risks of adverse reactions and potential transfusion-transmitted infections (TTIs). Detection of TTIs were not included in the study protocol, nor an extended follow up which may have detected TTIs[14] thus a potential limitation.

The other major users of the blood transfusion services in Africa are the maternity services for the emergency management of mothers with bleeding complications. Treatment of anaemia by packed cells does not replace the volume deficit, thus mothers require additional intravenous fluids in order to maintain their total blood volume. Transfusing whole blood could circumnavigate this additional requirement[23]. Together with the findings from the TRACT trial in children, this suggests that component preparation to produce packed or settled cells may not be the optimal strategy for the two largest user groups of transfusion services. In Zimbabwe per pack a whole blood transfusion is up to 16% cheaper than transfusion with packed cells[24], suggesting that transfusion with whole blood could lead to considerable savings. The current process of producing 'red cell concentrates' for paediatric and neonatal transfusion (pedipacks) could be more readily replaced, at the point of collection of blood from a donor, by directly splitting the donation this into a number smaller packs (whole blood pedipacks). This is now standard practice within the Malawi blood transfusion service (Supplying Blood for African Children: https://vimeo.com/540381518).

In conclusion, evidence from our study suggests that the use of packed or settled cells rather than whole blood leads to more additional transfusions; increasing the use of a relatively scarce resource in most sub-Saharan African countries. Whilst further consideration should be given by blood transfusion services as to how to streamline their requirements to include whole blood, when and where it is most needed[22], rather than a one-size fits all policy for all regional BTS centres, a clinical trial comparing whole blood transfusion to red cell concentrates maybe needed to convince policy makers.

Author's contributions

KM designed the study. SK PO-O, ROO, GC, FA and SU, gathered the data. ECG analysed the data. IB and DM hosted a meeting of blood transfusion specialists who reviewed the results of the TRACT trial and requested further analysis of pack type on outcome. KM and ECG wrote the article, which all authors commented on. All authors had access to the data in the study; ECG and ASW had access to and verify the data used in the study.

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Availability of data and materials

The data used in these analyses was collected as part of the TRACT trial, sponsored by Imperial College London, and is stored securely at MRC CTU at UCL. The datasets analysed during the current study are available from the corresponding author on reasonable request (k.maitland@imperial.ac.uk).

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Ethics Approval Statement

The ethics committees of Imperial College London, UK, Makerere University, Uganda, and the College of Medicine, Blantyre, Malawi, approved the trial protocol from which this further analysis has been conducted. Where prior written consent from parents/legal guardians could not be obtained, ethics committees approved verbal assent with delayed written informed consent as soon as practicable.

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Table 1: Baseline characteristics of children included in this analysis

	Type of first blood pack in initial transfusion				
Baseline characteristics at initial transfusion	**Whole blood pack N=1404,**	**Packed cells pack N=692,**	**Settled cells pack N=1092,**	**N=3188**	**p-value***
Median age (months) (IQR)	39 (22, 66)	37 (16, 70)	33 (16, 61)	37 (18, 64)	0.001
Mean age (months) sd	47 (33)	47 (36)	42 (32)	45 (33)	0.002
Child sex: Male	803 (57%)	395 (57%)	612 (56%)	1810 (57%)	0.83
Female	601 (43%)	297 (43%)	480 (44%)	1378 (43%)	
Median haemoglobin (g/dL) (IQR)	4.2 (3.4, 5.2)	4.3 (3.4, 5.3)	4.2 (3.3, 5.1)	4.2 (3.4, 5.2)	0.46
Haemoglobin <4g/dl	580 (41%)	288 (42%)	470 (43%)	1338 (42%)	0.67
Mean temperature (°C) (sd)	37.4 (1.0)	37.4 (1.0)	37.4 (0.9)	37.4 (1.0)	0.34
Mean heart rate (bpm) (sd)	146 (21)	145 (23)	148 (23)	146 (23)	0.008
Median oxygen saturation (%) (IQR)	98 (95, 99)	97 (95, 98)	97 (95, 99)	97 (95, 99)	0.001
Mean respiratory rate (brpm)[$] (sd)	42 (12)	45 (13)	44 (14)	44 (13)	0.0001
HIV status: positive	47 (4%)	35 (5%)	16 (2%)	98 (3%)	<0.0001
negative	1280 (96%)	636 (95%)	1026 (98%)	2942 (97%)	
Malaria status: positive	938 (67%)	375 (54%)	732 (67%)	2045 (64%)	<0.0001
negative	463 (33%)	316 (46%)	358 (33%)	1137 (36%)	
Blood culture: positive	39 (4%)	24 (4%)	29 (3%)	92 (3%)	0.70
negative	1071 (96%)	634 (96%)	949 (97%)	2654 (97%)	
Median CRP (mg/dL) (IQR)	63.7 (24.5, 112.0)	56.0 (18.5, 126.0)	62.4 (25.9, 113.0)	62.0 (23.8, 114.4)	0.22
Median lactate (mmol/L) (IQR)	2.9 (2.0, 4.9)	3.0 (2.1, 4.4)	2.7 (1.7, 4.5)	2.9 (1.9, 4.6)	0.04
Mean glucose (mmol/L) (sd)	5.8 (1.5)	5.4 (1.3)	5.7 (1.6)	5.7 (1.6)	<0.0001
Impaired consciousness: Yes	332 (24%)	216 (31%)	204 (19%)	752 (24%)	<0.0001
No	1072 (76%)	476 (69%)	888 (81%)	2436 (76%)	
Haemoglobinurea[§]: Yes	278 (20%)	97 (14%)	214 (20%)	589 (18%)	0.003
No	1126 (80%)	595 (86%)	878 (80%)	2599 (82%)	
Sickle genotype - AA	1035 (74%)	434 (63%)	713 (65%)	2182 (68%)	<0.0001
- AS	42 (3%)	22 (3%)	31 (3%)	95 (3%)	
- SS unknown	153 (11%)	148 (21%)	153 (14%)	454 (14%)	
- SS known	164 (12%)	72 (10%)	193 (18%)	429 (13%)	
Patient blood group - A	362 (26%)	171 (25%)	295 (27%)	828 (26%)	0.01
- B	345 (25%)	152 (22%)	288 (26%)	785 (25%)	
- AB	83 (6%)	25 (4%)	63 (6%)	171 (5%)	
- O	614 (44%)	343 (50%)	446 (41%)	1403 (44%)	
Mean pack haemoglobin (g/dl) (sd)	14.8 (3.3)	19.5 (2.5)	16.9 (2.8)	16.6 (3.5)	<0.0001
Mean pack haematocrit (%) (sd)	47.1 (13.8)	60.4 (8.4)	53.4 (11.2)	52.0 (13.0)	<0.0001
Mean pack age (days) (sd)	12 (9)	14 (9)	14 (8)	13 (9)	0.0003
Site - Blantyre, Malawi	318 (23%)	77 (11%)	0 (0%)	395 (12%)	<0.0001
Mulago, Uganda	70 (5%)	602 (87%)	60 (5%)	732 (23%)	
Soroti, Uganda	616 (44%)	0 (0%)	244 (22%)	860 (27%)	
Mbale, Uganda	400 (28%)	13 (2%)	788 (72%)	1201 (38%)	

CRP: C-reactive protein.

*p-value calculated from a chi-squared test for categorical variables, a K-sample equality-of-medians test for continuous variables comparing medians and an one-way ANOVA for comparing means.

[§]Red or cola coloured urine. [$] breaths per minute.

Table 2: Characteristics of the packs used in transfusions.

Pack characteristics	**Expected values for whole blood**	**Whole blood (N=1994 packs), median (IQR)**	**Expected values for red cell concentrates**	**Packed cells (spun) (N=968 packs), median (IQR)**	**Settled cells (gravity) (N=1589 packs), median (IQR)**	**Overall (N=4551 packs)**
Pack haemoglobin (g/dl)	>12	13.9 (12.6, 16.0)	15-20	19.5 (17.7, 21.1)	16.9 (15.0, 18.7)	16.3 (13.8, 19.0)
Pack haematocrit (%)	35-45	42.1 (38.0, 52.0)	50-70	60.0 (55.3, 65.0)	52.1 (45.4, 60.0)	50.9 (42.0, 60.0)
Pack age (days)	<36	10 (5, 18)	<43	12 (7, 20)	13 (7, 19)	12 (6, 19)

Table 3: Impact of blood pack type supplied for transfusion on clinical outcomes

Outcome	Adjusted estimate (95% CI) (packed cells vs whole blood packs)	Adjusted estimate (95% CI) (settled cells vs whole blood packs)	Overall Wald p-value
Time to event analyses (Hazard ratios)			
28 day mortality*	0.99 (0.48, 2.04)	1.12 (0.64, 1.95)	0.92
180 day mortality*	1.11 (0.66, 1.85)	1.05 (0.75, 1.46)	0.91
Readmissions - all cause§	1.05 (0.66, 1.65)	0.85 (0.68, 1.06)	0.30
Readmissions - anaemia§	1.49 (0.87, 2.57)	0.90 (0.69, 1.16)	0.18
Readmissions - DUS§	0.61 (0.12, 3.02)	1.08 (0.70, 1.67)	0.76
Readmissions - Malaria§	0.59 (0.25, 1.39)	0.70 (0.47, 1.02)	0.13
Time to discharge§	0.94 (0.81, 1.10)	0.86 (0.79, 0.94)	0.002
Change in haemoglobin analyses (g/dl)			
8 hour haemoglobin - 20mls/kg¥	-1.3 (-1.5, -1.0)	-1.1 (-1.2, -0.9)	<0.0001
8 hour haemoglobin - 30mls/kg¥	-1.4 (-1.6, -1.1)	-1.5 (-1.7, -1.3)	<0.0001
180 day haemoglobin¥	0.0 (-0.4, 0.4)	0.1 (-0.1, 0.2)	0.57
Second transfusions (Odds ratio)			
Odds of second transfusion°	2.32 (1.30, 4.12)	2.97 (2.18, 4.05)	<0.0001

DUS: Dark-urine syndrome (also known as haemoglobinurea)

All models were adjusted for site and each model checked for heterogeneity of effect by volume randomisation. The p-values from the heterogeneity tests (likelihood ratio test or Wald test on 2 degrees of freedom) on ten models were then ranked and compared with critical values from the Benjamin Hochberg procedure, to account for the multiple testing and strong evidence was only found in the model for change in haemoglobin at 8 hours (p=0.003, critical value p=0.005) and thus results were presented separately by volume randomisation for this model. All other models had p-values above their corresponding critical value and thus effect estimates were presented overall.

. The Wald tests are on 2 degrees of freedom comparing both adjusted estimates to the null.

*Cox regression models (see supplementary material). There was no evidence of non-proportionality in the effect of pack type for mortality outcomes (Schoenfeld p>0.05).

§Competing risks regression models. For readmission the competing event was death post-discharge, for time to discharge it was death during initial admission (see supplementary material).

¥Linear regression models (see supplementary material).

°Logistic regression model (see supplementary material).

Figure 1: Haemoglobin recovery over time by a) type of first blood pack in first transfusion; b) age of first blood pack in first transfusion; c) haemoglobin of first blood pack

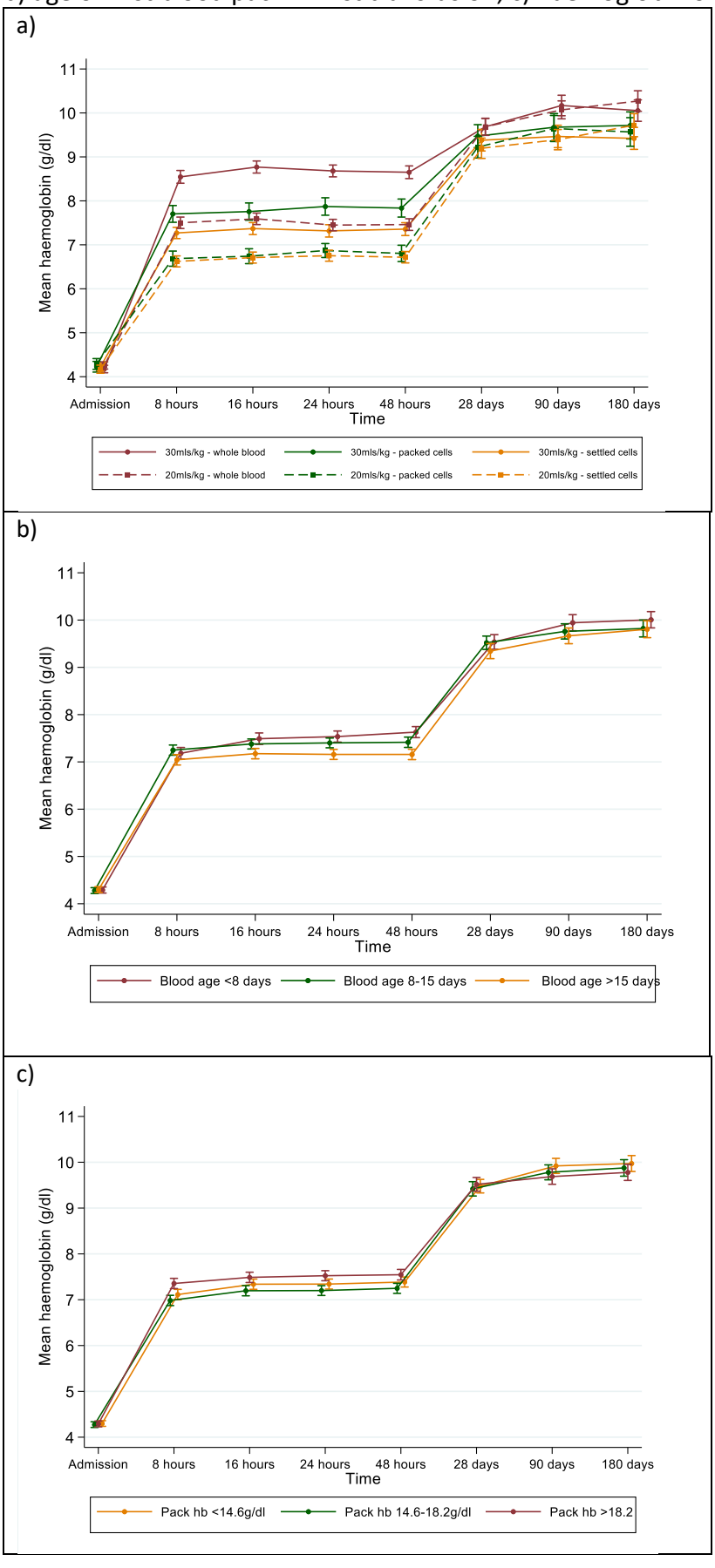
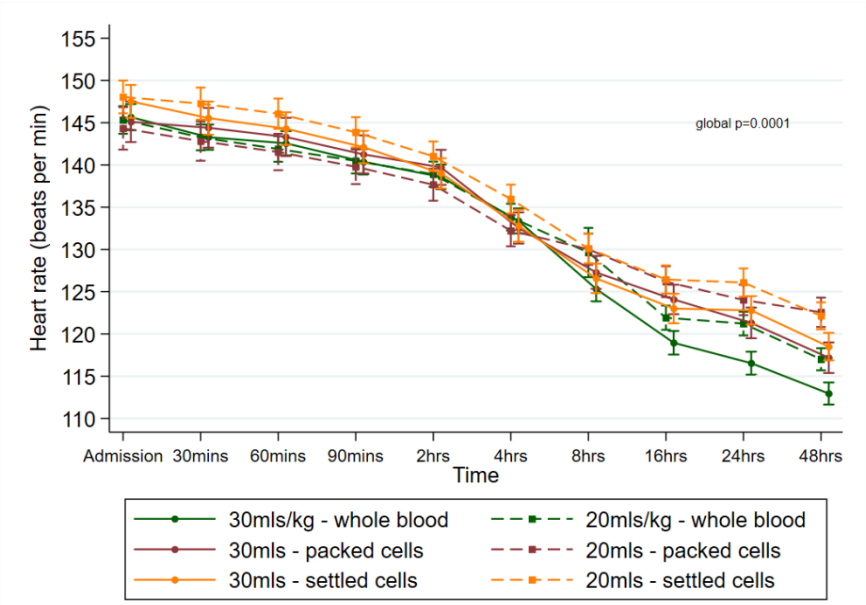


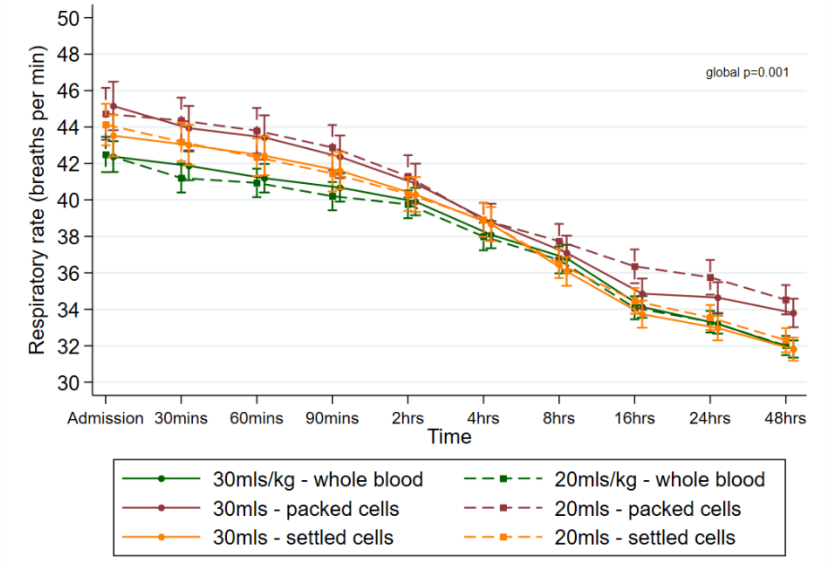
Figure 2: Heart rate and respiratory rate over time from beginning of first transfusion

a) Heart rate



Global p-value for differences between pack types estimated by GEE model over all time points adjusting for baseline values.

b) Respiratory Rate



Global p-value for differences between pack types estimated by GEE model over all time points adjusting for baseline values.