RESEARCH ARTICLE

REVISED Informing thresholds for paediatric transfusion in Africa: the need for a trial [version 2; peer review: 2 approved]

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Abstract

Background: Owing to inadequate supplies of donor blood for transfusion in sub-Saharan Africa (sSA) World Health Organization paediatric guidelines recommend restrictive transfusion practices, based on expert opinion. We examined whether survival amongst hospitalised children by admission haemoglobin and whether this was influenced by malaria infection and/or transfusion.

Methods: A retrospective analysis of standardised clinical digital records in an unselected population of children admitted to a rural hospital in Kenya over an 8-year period. We describe baseline parameters with respect to categories of anaemia and outcome (in-hospital death) by haemoglobin (Hb), malaria and transfusion status.

Results: Among 29,226 children, 1,143 (3.9%) had profound anaemia (Hb <4g/dl) and 3,469 (11.9%) had severe anaemia (Hb 4-6g/d). In-hospital mortality rate was 97/1,143 (8.5%) if Hb<4g/dl or 164/2,326 (7.1%) in those with severe anaemia (Hb ≥4.0-<6g/dl). Admission Hb <3g/dl was associated with higher risk of death versus those with higher Hbs (OR=2.41 (95%CI: 1.8 - 3.24; P<0.001), increasing to OR=6.36, (95%CI: 4.21–9.62; P<0.001) in malaria positive children. Conversely, mortality in non-malaria admissions was unrelated to Hb level. Transfusion was associated with a non-significant improvement in outcome if Hb<3g/dl (malaria-only) OR 0.72 (95%CI 0.29 - 1.78), albeit the number of cases were too few to show a statistical difference. For those with Hb levels above 4g/dl, mortality was significantly higher in those receiving a transfusion compared to the non-transfused group. For non-malarial cases, transfusion did not affect survival-status, irrespective of baseline Hb level compared to children who were not transfused at higher Hb levels.

Conclusion: Although severe anaemia is common among children admitted to hospital in sSA (~16%), our data do not indicate that outcome is improved by transfusion irrespective of malaria status. Given the limitations of observational studies, clinical trials investigating the role of transfusion in outcomes in children with severe anaemia are warranted.

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
REVISED **version 2** published 12 Aug 2019	✓ report	✓ report
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Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Severe Anaemia, Children, Kenya, Africa, Mortality, Transfusion, Sepsis, Malaria, Guidelines

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REVISED **Amendments from Version 1**

I have addressed the reviewer comments and also been able to reference the published TRACT manuscripts which addresses some of the concerns raised

See referee reports

Abbreviations

Hb Haemoglobin; KCH Kilifi County Hospital; mps malaria parasites; MUAC mid-upper arm circumference; RCT randomised controlled trial; OR Odds ratios; rbc red blood cells; sSA sub-Saharan Africa; wbc white blood cells; WHZ Weight for Height Z score; WHO World Health Organization.

Introduction

Transfusion of blood can be a life-saving intervention, and provision of adequate supplies of safe blood for transfusion are an essential undertaking for any health system. Issues of blood safety, adequate supply, equitable access and rational use, however, remain key challenges throughout the world. In resource-limited countries in sub-Saharan Africa (sSA) these issues present major barriers to the development of successful nationally-coordinated blood transfusion services[1]. The World Health Organization (WHO) Global Database on Blood Safety reports a mismatch between supply and demand. For example, the 2016 WHO survey found that of the 46 countries reporting from the WHO African Region, (which are home to approximately 13% of the global population) overall they collected a total of approximately 5.6 million blood donations, which accounted for only 4% of global donations[2]. In an earlier report the average blood donation rate was 2.3/1000 population in countries with a low human development index in comparison to 36.7 in countries with a high human developmental index (HDI)[2,3]. The figure for the Africa Region (excluding South Africa) is only 3.4/1000 compared to the WHO estimated optimal requirement of 10-20/1000[4].

In order to bridge the major gap between supply and demand, one of the four key goals, mandated in a WHO resolution on an integrated strategy of blood safety in 1975 was to 'reduce unnecessary transfusions' through the more effective clinical use of blood and the use of simple alternatives to transfusion (such as crystalloids and colloids) where possible[5]. WHO has subsequently developed and published guidelines for the appropriate use of blood for patient groups suffering the greatest supply shortages[6,7]. Notably, the pattern of blood utilisation in sSA is very different from that in high HDI nations, where elective-use predominates and where supply is strictly monitored through specialist transfusion services. By contrast, the 2016 WHO survey on world safety and availability of blood transfusions found that in low-and-middle-income countries, 67% of transfusions are received by children under 5 years old, followed by women for pregnancy-related complications[2] with most being given as emergency interventions[8].

What is already known?

For paediatric transfusion, the WHO conservative policy reserves blood transfusion for children with a Hb <4g/dl or for those with an Hb <6g/dl if accompanied by life-threatening complications[6,7]. These specific recommendations have not been systematically evaluated. Consequently, compliance is often poor and many children receive unwarranted transfusions[9,10]. Nevertheless, adverse outcomes following admission to hospital with severe anaemia in children are common, with case-fatality rates being high both within-hospital (9–10%)[11] and within 6-months of discharge (12%)[12], in common with rates of relapse or re-hospitalisation (6%)[12]. Such data suggest that current recommendations and management strategies may not be working in practice. Although the conservative WHO transfusion guidelines were developed to protect scarce resources, little research has since been conducted to either support or challenge the haemoglobin thresholds for administering a transfusion. With this in mind, we have conducted a retrospective analysis of mortality outcome by Hb level at admission in an unselected paediatric population admitted to a rural district hospital in Kenya over an 8-year period. Secondary aims were to examine whether outcome (survival) was influenced by malaria infection and/or transfusion.

Methods

The study was conducted on the paediatric wards at Kilifi County Hospital (KCH), a rural district hospital on the coast of Kenya, where a system of routine surveillance has been operated by the KEMRI-Wellcome Trust Research Programme since 1989. All children <15 years of age on admission to KCH were assessed by a clinician. Standardised clinical proforma are entered directly onto a computerized database. In addition, all patients were investigated with a standard set of laboratory tests including a full blood count, a blood culture and a blood film for malarial parasites. HIV status was tested following parental assent. At discharge (or in fatal cases) the clinician completed a standard summary onto the computerized database which included discharge diagnosis and whether a blood transfusion had been given. Blood transfusion policy at KCH follows WHO guidelines.

All admission records for the 8-year period January 2002 to September 2009 were included in the current analysis. Throughout this period a single method for Hb measurement (Coulter counter, Coulter Electronics) was used thus minimising potential methodological variation. Clinical data on key variables were retrieved together with the key co-morbidities, including bacteraemia, malarial parasitaemia and nutritional and HIV status, the receipt of a whole blood transfusion during admission and discharge status (alive or dead).

Data analysis

Our analysis included data from 36,621 consecutive admissions to the KCH paediatric ward (See Figure 1: Study Flow). Patients with missing data on the primary exposure (admission Hb); 1,482 observations, 4.0%) or on the primary outcome (in-hospital mortality as defined by status at discharge; 208 observations, 0.1%) were excluded from the analysis. Infants under 60 days (6,285 observations, 17%) and cases with a Hb well outside the normal range, >19.0 g/ld. (240, 0.1%) were excluded. With these adjustments, 29,226 patients remained for analysis. Z-scores for the anthropometric parameters weight-for-age (WAZ) were calculated for each individual using Epi Info v2000 (CDC, Atlanta) and undernutrition defined as a WAZ of <-3 while severe malnutrition was defined as a mid-upper arm circumference (MUAC) of <11.5cm. Dichotomous and categorical variables were created from continuous variables. Shock was

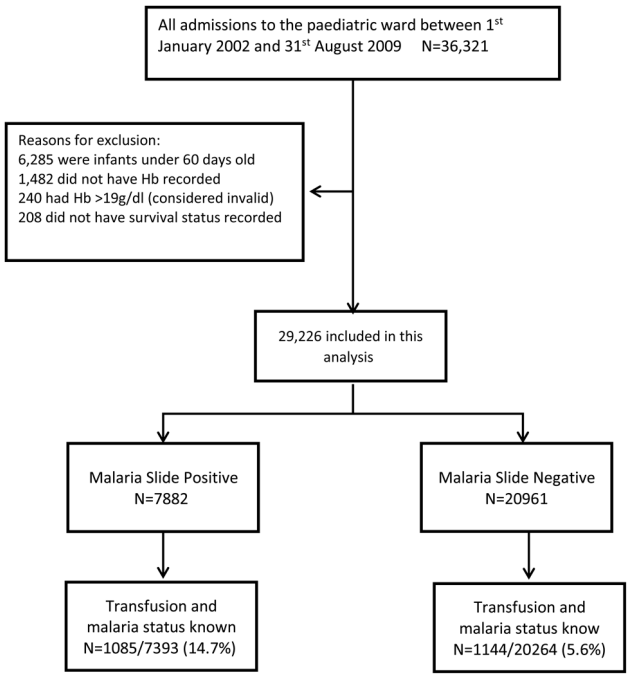

Figure 1. Study flow.

defined on the basis of one or more of the following clinical features: capillary refill time >2 seconds; weak pulse or a temperature gradient in the lower limbs (defined as a temperature cline detected when running the back of the hand down from thigh to shin). We also examined whether the clinicians were compliant with WHO guidelines for transfusion. Compliance was considered using only the features from admission. The WHO restricts transfusion to those with Hb <4g/dl or for those with an Hb <6g/dl if accompanied by life-threatening complications or severity features (any one of shock (any feature of delayed capillary refilling time more than 2 seconds, weak pulse or cold extremities (temperature gradient, severe dehydration (sunken eyes or decreased skin turgor), impaired consciousness (prostration or coma (inability to localise a painful stimulus), respiratory distress or high parasitaemia at admission).

Odds ratios (OR) for mortality in patients with an Hb below (exposed group) versus those with an Hb equal to or above (unexposed) specific thresholds, determined depending on the presence or absence of additional clinical features, were assessed using logistic regression and adjusted for malaria and transfusion status to examine the risk of in-hospital death by level of Hb at admission. All analyses were conducted using STATA Version 11 (Stata Corporation, Texas, USA).

Results

A total of 29,226 patients were included for analysis. Baseline characteristics are described by age group (Table 1) and by the severity of anaemia (Table 2). At admission, 1,143 (3.9%) had profound anaemia (Hb <4g/dl); 2,326 (7.1%) had severe anaemia (Hb 4- < 6.0 g/dl) and almost one third (9,457) had moderate anaemia (6.0-8.9 g/dl). The admission cohort included 5,914 (20.2%) children with malnutrition described by weight for age <-3; alternatively 4,175/28,734 (14.6%) by mid-upper arm circumference (MUAC) <11.5 cm. The majority of children (58.8%) were febrile (axillary temperature >37.%C: measured by digital thermometer) at presentation. Clinical pallor defined by examination of conjunctiva, nail bed and palms of hands or soles of feet was present in 95% of children with profound anaemia (Hb<4g/dl); 77% with Hb 4-6g/dl, and 19% of children Hb>6g/dl (Table 2); jaundice was uncommon (1.9%).

Deep 'acidotic' breathing and/or indrawing (respiratory distress) were present in 3,104/29,180 (10.6%) and 7,338/29,183 (25.1%), respectively. Respiratory distress was associated with higher case fatalities 487/3104 (15.7%) and 575/7,338 (7.8%), respectively, compared to 1053/26,076 (4.0%) or 966/21,845 (4.4%) in children without either of these signs. Notably few had hypoxaemia (defined as a pulse oximetry (pSO_2) reading of less that 90%) present in on 118/1143 (10.3%) in children with profound anaemia. Clinically-defined shock was present in 4,879 (16.7%) and was associated with a higher fatality 612 (12.5%) compared to those without shock 927/24,300 (3.8%). Overall, 1,249/27909 (4.5%) children had culture-proven bacteraemia, with a case fatality exceeding 20% (Table 1). In children with profound anaemia and severe anaemia 47% and 53% were malaria-parasite positive; however overall mortality was lower, relative to other co-morbidities with 280 deaths (case fatality rate, 3.6%). HIV status (antibody) data are less reliable owing to the large number of missing data (17,974 (61.5%)).

Compliance with WHO guidelines

Overall, compliance with WHO transfusion guidelines was good, especially with respect to transfusions given to children with an admission Hb >6g/dl, which occurred in only 520/24372 (2.1%). For those with Hb< 4gdl (n=1134), 912 (80%) were transfused (Table 3). Children with an admission Hb 4-6g/dl, which included 1629/27,904 (5.8%) of children at hospital admission, 1564 (5.6%) of all admissions met the severity criteria to transfuse, but only 567 (36.3%) were transfused and 746 did not met severity criteria yet 194 (26% of this category) were transfused (Table 3). The reasons for transfusion or no transfusion were not systematically captured on the database.

Mortality by severity of anaemia

Profound anaemia (Hb<4g/dl) was associated with the highest fatality 97/1,143(8.5%) compared to 164/2,326 (7.1%) with severe anaemia (Hb ≥4.0-<6g/dl); 627/9,457 (6.6%) with moderate anaemia (≥6.0-<9.0 g/dl) and 660/16,300 (4.0%) without anaemia (Hb≥9g/dl) (Table 1). The increased risk of mortality in comparison to children with a higher admission Hb level was greatest (OR=1.70; 95%CI: 1.37-2.11) among children with profound anaemia (Hb<4g/dl). The strength of this association was less apparent when comparing the probability of death across the anaemia subgroups, for example profound anaemia vs severe anaemia (P=0.13) and in severe anaemia vs moderate anaemia (P=0.80).

We therefore conducted an in-depth examination of the risk of fatal outcome for each stratum of Hb level compared to all Hb's above that stratum in an attempt to validate the current haemoglobin thresholds for transfusion (Figure 2). Overall, children with a Hb of <3g/dl were at significantly higher risk of death

Table 1. Baseline variables by age group.

	>2 to <12 months (n=8624)		12 to <24 months (n=7189)		24 to <60 months (n=8637)		>= 60 months (n=4776)		TOTAL (n=29226)	
	Freq. (%)	Died (%)	Freq. (%)	Died (%)	Freq. (%)	Died (%)	Freq. (%)	Died (%)	Freq. (%)	Died (%)
Discharge status										
Alive	8121 (94.1)	---	6847 (95.2)	---	8194 (94.9)	---	4516(94.5)	---	27678 (94.7)	---
Dead	503 (5.9)	---	342 (4.8)	---	443 (5.1)	---	260 (5.5)	---	1548 (5.3)	---
Hb at admission										
<4.0 g/ld.	254 (3.0)	20 (7.9)	285 (4.0)	21 (7.4)	381 (4.1)	39 (10.2)	223 (4.7)	17 (7.6)	1143 (3.9)	97 (8.5)
4.0 to <6.0 g/ld.	596 (6.9)	35 (5.9)	648 (9.0)	31 (4.8)	766 (8.9)	56 (7.3)	316 (6.6)	42 (13.3)	2326 (8.0)	164 (7.1)
6.0 to <9.0 g/ld.	2880 (33.4)	221 (7.7)	2701 (37.6)	161 (6.0)	2781 (32.2)	175 (6.3)	1095 (22.9)	70 (6.4)	9457 (32.4)	627 (6.6)
9.0 to <12.0 g/ld.	4593 (53.3)	199 (4.3)	3379 (47.0)	118 (3.5)	4092 (47.4)	146 (3.6)	2300 (48.2)	86 (3.7)	14364 (49.1)	549 (3.8)
>= 12.0 g/ld.	301 (3.5)	28 (9.3)	176 (2.4)	11 (6.3)	617 (7.1)	27 (4.4)	842 (17.6)	45 (5.3)	1936 (6.6)	111 (5.7)
Sex										
Female	3723 (43.2)	247 (6.6)	3166 (44.0)	160 (5.1)	3849 (44.6)	198 (5.1)	2120 (44.4)	112 (5.3)	12858 (44.0)	717 (5.6)
Male	4901 (56.8)	256 (5.2)	4023 (56.0)	182 (4.5)	4788 (55.4)	245 (5.1)	2656 (55.6)	148 (5.6)	16368 (56.0)	831 (5.1)
WAZ score <-3										
No	7360 (85.3)	297 (4.0)	5112 (71.1)	93 (1.8)	6628 (76.7)	192 (2.9)	3996 (83.7)	168 (4.2)	23096 (79.0)	750 (3.2)
Yes	1237 (14.3)	199 (16.1)	2049 (28.5)	242 (11.8)	1954 (22.6)	237 (12.1)	674 (14.1)	82 (12.2)	5914 (20.2)	760 (12.9)
Missing value	27 (0.3)	---	28 (0.4)	---	55 (0.6)	---	106 (2.2)	---	216 (0.7)	---
MUAC (<11.5 cm)										
No	6358 (73.7)	192 (3.0)	5953 (82.8)	137 (2.3)	7746 (89.7)	263 (3.4)	4482 (93.8)	200 (4.5)	24539 (84.0)	792 (3.2)
Yes	2142 (24.8)	266 (12.4)	1150 (16.0)	189 (16.4)	721 (8.4)	131 (18.2)	182 (3.8)	37 (20.3)	4195 (14.4)	623 (14.9)
Missing value	124 (1.4)	---	86 (1.2)	---	170 (2.0)	---	112 (2.4)	---	492 (1.7)	---
Febrile (>37.5°C)										
No	3403 (39.5)	97 (2.9)	2861 (39.8)	113 (3.9)	3374 (39.1)	101 (3.0)	2350 (49.2)	61 (2.6)	11988 (41.0)	372 (3.1)
Yes	5209 (60.4)	405 (7.8)	4313 (60.0)	228 (5.3)	5248 (60.8)	339 (6.5)	2412 (50.5)	198 (8.2)	17182 (58.8)	1170 (6.8)
Missing value	12 (0.1)	---	15 (0.2)	---	15 (0.2)	---	14 (0.3)	---	56 (0.2)	---
Deep breathing										
No	7362 (85.4)	316 (4.3)	6400 (89.0)	228 (3.6)	7798 (90.3)	305 (3.9)	4516 (94.6)	204 (4.5)	26076 (89.2)	1053 (4.0)
Yes	1245 (14.4)	185 (14.9)	780 (10.9)	113 (14.5)	827 (9.6)	135 (16.3)	252 (5.3)	54 (21.4)	3104 (10.6)	487 (15.7)
Missing value	17 (0.2)	---	9 (0.1)	---	12 (0.1)	---	8 (0.2)	---	46 (0.2)	---

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	>2 to <12 months (n=8624)		12 to <24 months (n=7189)		24 to <60 months (n=8637)		>= 60 months (n=4776)		TOTAL (n=29226)	
Indrawing										
No	4890 (56.7)	214 (4.4)	5474 (76.1)	240 (4.4)	7262 (84.1)	324 (4.2)	4219 (88.3)	188 (4.5)	21845 (74.8)	966 (4.4)
Yes	3724 (43.2)	287 (7.7)	1705 (23.7)	101 (5.9)	1359 (15.7)	116 (8.5)	550 (11.5)	71 (12.9)	7338 (25.1)	575 (7.8)
Missing value	10 (0.1)	---	10 (0.1)	---	16 (0.2)	---	7 (0.2)	---	43 (0.2)	---
Delayed CRF (>=3 s)										
No	8030 (93.1)	369 (4.6)	6758 (94.0)	260 (3.8)	8101 (93.8)	325 (4.0)	4548 (95.3)	200 (4.4)	27437(93.9)	1154 (4.2)
Yes	579 (6.7)	130 (22.5)	416 (5.8)	81 (19.5)	515 (6.0)	112 (21.7)	209 (4.4)	54 (25.8)	1719 (5.9)	377 (21.9)
Missing value	15 (0.2)	---	15 (0.2)	---	21 (0.2)	---	19 (0.4)	---	70 (0.2)	---
WHO shock definition										
No	7119 (82.6)	300 (4.2)	5931 (82.5)	199 (3.4)	7125 (82.5)	264 (3.7)	4125 (86.4)	164 (4.0)	24300 (83.2)	927 (3.8)
Yes	1500 (17.4)	202 (13.4)	1246 (17.3)	142 (11.4)	1498 (17.3)	176 (11.7)	635 (13.3)	92 (14.5)	4879 (16.7)	612 (12.5)
Missing value	5 (0.1)	---	12 (0.2)	---	14 (0.2)	---	16 (0.3)	---	47 (0.2)	---
Coma (BCS <= 2)										
No	8258 (95.8)	387 (4.7)	6856 (95.4)	281 (4.1)	7904 (91.5)	295 (3.7)	4517 (94.6)	204 (4.5)	27535 (94.2)	1167 (4.2)
Yes	351 (4.1)	114 (32.5)	321 (4.5)	59 (18.4)	720 (8.3)	145 (20.1)	244 (5.1)	55 (22.5)	1636 (5.6)	373 (22.8)
Missing value	15 (0.2)	---	12 (0.2)	---	13 (0.2)	---	15 (0.3)	---	55 (0.2)	---
Bacteraemia										
No	7968 (92.4)	383 (4.8)	6656 (92.6)	285 (4.3)	7993 (92.5)	362 (4.5)	4139 (86.7)	197 (4.8)	26756 (91.6)	1227 (4.6)
Yes	422 (4.)	106 (25.1)	291 (4.1)	55 (18.9)	296 (3.4)	70 (23.6)	249 (5.2)	51 (20.5)	1258 (4.3)	282 (22.4)
Missing value	234 (2.7)	---	242 (3.4)	---	348 (4.0)	---	388 (8.1)	---	1212 (4.1)	---
Malaria Parasites										
No	7304 (84.7)	460 (6.3)	5207 (72.4)	275 (5.3)	5043 (58.4)	294 (5.8)	3521 (73.7)	221 (6.3)	21075 (72.1)	1250 (5.9)
Yes	1247 (14.5)	37 (3.0)	1920 (26.7)	62 (3.2)	3526 (40.8)	145 (4.1)	1202 (25.2)	36 (3.0)	7895 (27.0)	280 (3.5)
Missing value	73 (0.8)	---	62 (0.9)	---	68 (0.8)	---	53 (1.1)	---	256 (0.9)	---
HIV positive*										
No	2050 (93)	100 (4.9)	1751 (92.7)	71 (4.1)	1881 (91.0)	63 (3.3)	1205 (90.9)	43 (3.6)	6887 (92.1)	277 (4.0)
Yes	147 (6.7)	33 (22.4)	138 (7.3)	23 (16.7)	187 (9.0)	33 (17.6)	121 (9.1)	20 (16.5)	593 (7.9)	109 (18.4)

*Excluding cases that were not tested, discordant, and missing observations (number excluded = 21746 (74.4%))

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Table 2. Baseline characteristics by severity of anaemia.

Variable	Hb <4g/dl n (%)	Hb 4 to <6 g/dl n (%)	Hb ≥6/dl n (%)	Total n (%)
N (%)	N = 1143 (3.9)	N =2326 (8.0)	N = 25,757 (88.1)	N = 29226
Age Group (months) 2–11 m 12–23m 24–59m ≥60 m	 254 (22.2) 285 (24.9) 381 (33.3) 223 (19.5)	 596 (25.6) 648 (27.9) 766 (32.9) 316 (13.6)	 7774 (30.2) 6256 (24.3) 7490 (29.1) 4237 (16.5)	 8624 (29.5) 7189 (24.6) 8637 (29.6) 4776 (16.4)
Sex Males (%)	603 (52.8)	1282 (55.1)	14483 (56.2)	16368 (56.0)
Fever: axillary temp ≥37.5C	591 (51.8)	1454 (62.5)	15137 (58.8)	17182 (58.8)
Pallor	1086 (95.0)	1796 (77.2)	4967 (19.3)	7849 (26.9)
Visable Jaundice	55 (4.8)	124 (5.3)	370 (1.4)	549 (1.9)
WAZ ≤ -3[a]	354 (31.0)	667 (28.7)	4893 (19.0)	5914 (20.2)
MUAC ≤11.5 cm[b]	213 (18.6)	427 (18.4)	3555 (13.8)	4195 (14.4)
Weak pulse volume	163 (14.3)	143 (6.2)	1051 (4.1)	1357 (4.6)
Tachycardia[13]	115 (10.1)	412 (17.7)	3884 (15.1)	4411 (15.1)
Capillary refill time ≥3s	300 (26.3)	283 (12.2)	1136 (4.4)	1719 (5.9)
Temperature gradient	245 (21.4)	419 (18.0)	3563 (13.8)	4227 (14.5)
Strict shock definition[c]	35 (3.1)	13 (0.6)	94 (0.4)	142 (0.5)
Severe dehydration[d]	81 (7.1)	200 (8.6)	3967 (15.4)	4248 (14.5)
Indrawing	279 (24.4)	545 (23.4)	6514 (25.3)	7338 (25.1)
Deep breathing[e]	252 (22.1)	350 (15.1)	2502 (9.7)	3104 (10.6)
Hypoxaemia(pulse oximetry) Oxygen saturation <90%	118 (10.3)	138 (5.9)	1232 (4.8)	1488 (5.1)
Base excess <-8[f]	383 (52.2)	547 (35.1)	5632 (39.9)	6562 (40.0)
Conscious level[g] Alert/Normal Prostration/Coma	 809 (83.8) 157 (16.3)	 1739 (87.1) 257 (12.9)	 20998 (94.3) 1274 (5.7)	 23546 (93.3) 1688 (6.7)
High malaria parasitaemia[h]	539 (47.2)	1252 (53.8)	6104 (23.7)	7895 (27.0)
HIV antibody positive[i]	17/223 (7.6)	52/446 (11.7)	524/6811 (7.7)	593/7480 (7.9)
Pathogenic bacterial isolate	63 (5.5)	172 (7.4)	1023 (4.0)	1258 (4.3)

[a]WAZ: weight for age Z-score

[b]MUAC: mid-upper arms circumference

[c]Presence of weak pulse volume & capillary refill time ≥3s & temperature gradient

[d]Presence of sunken eyes of decreased skin turgor

[e]Kussmaul's breathing

[f]Excludes missing observations (for base excess: number of missing observations = 12819 (43.9%), for conscious level: number of missing observations = 3992 (13.7%)

[g]Prostration: inability to sit upright (if >8months) or feed; coma failure to localise a painful stimulas

[h]hyperparasitaemia defined as:

percentage_parasitaemia= (malaria parasites (mps) per 100 white blood cells (wbc)/100)*wbc*1000 >10, or as
percentage_parasitaemia= (mps500 red blood cells (rbc)/500)*rbc*1000000 >10

[i]Excluding cases that were not tested, discordant, and missing observations (number excluded = 21746 (74.4%))

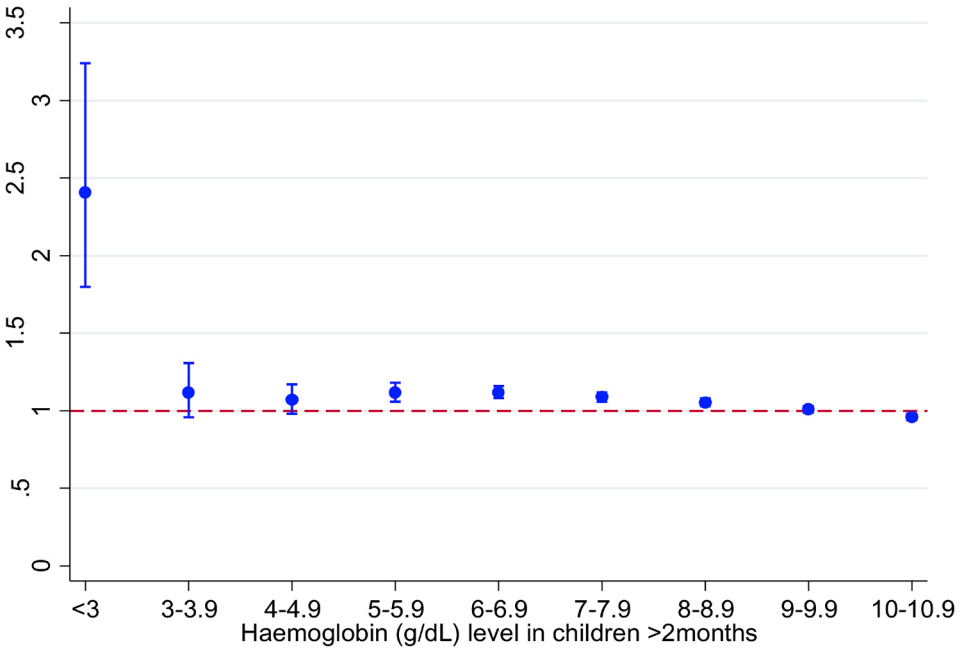
Table 3. Receipt of transfusion according to World Health Organization transfusion (WHO) criteria.

WHO Criteria	Category	Freq. (%)	Transfused (%)	Numbers transfused in sub-category (%)
Eligible for transfusion	Hb < 4	1134 (4.1)	912 (80.4)	1479 (54.8)
	Hb 4-6: Criteria +ve	1564 (5.6)	567 (36.3)	
Ineligible for transfusion	Hb 4-6: Criteria -ve	746 (2.7)	194 (26.0)	714 (2.8)
	Hb > 6	24372 (87.3)	520 (2.1)	

Criteria +ve includes any one of

- Clinically detectable dehydration
- Shock (compensated)
- Impaired consciousness
- Deep breathing
- Very high parasitaemia

Criteria – ve includes none of the above



Hb (g/dl)	<3	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9	>=11
Sample total	445	698	1023	1303	1918	3016	4523	5817	5329	5154
Cumulative frequency	445	1143	2166	3469	5387	8403	12926	18743	24072	29226
Cumulative %	1.52	3.91	7.41	11.87	18.43	28.75	44.23	64.13	82.37	100.0
Died	52	45	64	100	155	214	258	245	171	244
% Mortality	11.7	6.4	6.3	7.7	8.1	7.1	5.7	4.2	3.2	4.7
Odds Ratio	2.41	1.12	1.07	1.12	1.12	1.09	1.05	1.01	0.96	-
95% CI	1.80-3.24	0.96-1.31	0.98-1.17	1.06-1.18	1.08-1.16	1.06-1.12	1.03-1.08	0.99-1.03	0.94-0.98	
P	<0.001	0.13	0.11	<0.001	<0.001	<0.001	<0.001	0.43	<0.001	

Figure 2. Odds of mortality by haemoglobin (Hb) level status.

compared to those with a higher admission Hb (OR= 2.41 (95%CI: 1.8 - 3.24; P<0.001). Conversely, the risk of death with a Hb 3-3.9g/dl compared to those with a higher Hb was less pronounced OR=1.12 (95%CI: 0.96 - 1.31; P=0.13). Similarly, children who fall within the classification of severe or moderately-severe anaemia had very little variation in their risk of mortality across the whole range of haemoglobins from 4.0 to 9.9g/dl.

Outcome in relation to malaria

We analysed the risk of mortality separately for children with *Plasmodium falciparum* malaria versus non-malarial (blood slide-negative) admissions (Table 4a, Table 4b and Figure 3). There were substantial differences in the patterns of risk by haemoglobin level. Among children with malaria parasitaemia, the greatest risk of death was at Hb's of <3g/dl (OR=6.36, 95%CI: 4.21–9.62) compared to children with higher Hb's, whereas the risk was less marked at an Hb level of 3-3.9g/dl compared to children with higher haemoglobins (OR=1.33, 95%CI: 1.06–1.69; P=0.02). Mortality was only marginally increased across Hb values between 4 and 5.9.

The picture was different in those without malaria parasites in which risk of mortality was not clearly related to the severity of anaemia - mortality in those with Hb <3g/dl was 22/271 (8.1%) rising to 126/1052 (12%) in those with Hb 6.0-6.9 g/dl. The risk of mortality was greatest in those with Hb levels between 5-9.9g/dl.

Table 4a. Children with malaria: Odds of death at each level of haemoglobin (Hb) in those who received transfusion versus no transfusion.

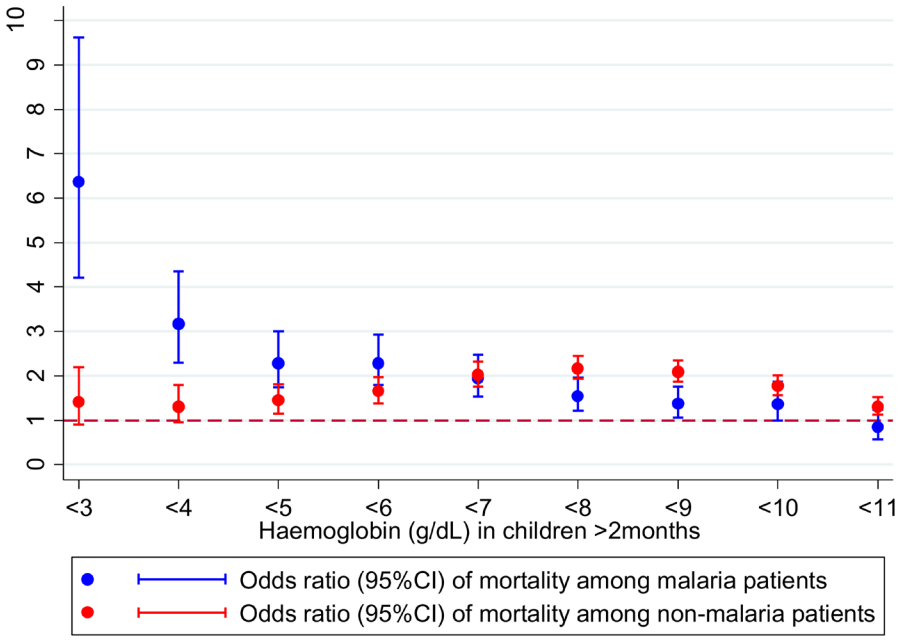
Hb level	N	Died	Not transfusion	Deaths non-transfused	Case fatality	Transfused	Deaths transfused	Case fatality	Odds ratio (95% CI)	P value
<3	169	29	38	8	21.1%	131	21	16.0%	0.72 (0.29 - 1.78)	0.471
3-3.9	366	20	62	5	8.1%	304	15	4.9%	0.59 (0.21 - 1.69)	0.328
4-4.9	552	24	250	5	2.0%	302	19	6.3%	3.29 (1.21 - 8.94)	0.02
5-5.9	657	36	479	16	3.3%	178	20	11.2%	3.66 (1.85 - 7.24)	<0.001
6-6.9	795	25	721	16	2.2%	74	9	12.2%	6.10 (2.59 - 14.35)	<0.001
7-7.9	948	20	911	17	1.9%	37	3	8.1%	4.64 (1.30 - 16.59)	0.018
8-8.9	1123	31	1,099	29	2.6%	24	2	8.3%	3.35 (0.75 - 14.94)	0.112
9-9.9	1186	34	1,169	28	2.4%	17	6	35.3%	22.23 (7.68 - 64.35)	<0.001
10-10.9	941	15	930	15	1.6%	11	0	0.00%	-	
>=11	656	22	649	21	3.2%	7	1	14.3%	4.98 (0.57 - 43.27)	0.145
Total	7393	256	6308	160	2.5%	1085	96	8.8%		

CI confidence interval

Table 4b. Children without malaria: Odds of death at each level of haemoglobin (Hb) in those who received transfusion versus no transfusion.

Hb level g/dl	N	Died	Not transfusion	Deaths non-transfused	Case fatality	Transfused	Deaths transfused	Case fatality	Odds ratio (95% CI)	P value
<3	266	20	54	4	7.4%	212	16	7.55%	1.02 (0.33 - 3.19)	0.972
3-3.9	323	23	64	5	7.8%	259	18	6.95%	0.88 (0.31 - 2.47)	0.81
4-4.9	436	40	214	15	7.0%	222	25	11.26%	1.68 (0.86 - 3.29)	0.127
5-5.9	586	61	483	34	7.0%	103	27	26.21%	4.69 (2.68 - 8.22)	<0.001
6-6.9	1013	121	931	85	9.1%	82	36	43.90%	7.79 (4.77 - 12.71)	<0.001
7-7.9	1884	180	1,815	142	7.8%	69	38	55.07%	14.44 (8.72 - 23.91)	<0.001
8-8.9	3154	209	3,090	173	5.6%	64	36	56.25%	21.68 (12.93 - 36.36)	<0.001
9-9.9	4316	192	4,267	169	3.96%	49	23	46.94%	21.45 (11.99 - 38.38)	<0.001
10-10.9	4115	142	4,074	129	3.2%	41	13	31.71%	14.20 (7.19 - 28.05)	<0.001
>=11	4171	193	4,128	174	4.2%	43	19	44.19%	17.99 (9.67 - 33.47)	<0.001
Total	20264	1181	19120	930	4.9%	1144	251	21.9%		

Diagnoses for those with Hb 8.0g and above (tables 3a and b) who were transfused:
Congenital abnormality (5); cerebral palsy (3); gastroenteritis (18); hepatitis (3), HIV (22); hypersplenism (1); Lower respiratory tract infection (18); malaria (45); malignancies (15); malnutrition (56); poisoning (5); renal failure (7); sickle cell disease (14); sepsis (23); snake bite (1); surgical (7); trauma (5) and unknown (6).



Malaria slide positive										
Hb (g/dl)	**<3**	**3-3.9**	**4-4.9**	**5-5.9**	**6-6.9**	**7-7.9**	**8-8.9**	**9-9.9**	**10-10.9**	**>=11**
Sample total	171	368	571	681	854	1019	1212	1297	994	728
Cumulative n	171	539	1110	1791	2645	3664	4876	6173	7167	7895
Cumulative %	2.2	6.8	14.1	22.7	33.5	46.4	61.8	78.2	90.8	100.0
Died	30	20	24	36	27	22	33	40	18	30
% mortality	17.5	5.4	4.2	5.3	3.2	2.2	2.7	3.1	1.8	4.1
Odds ratio	6.36	1.33	1.12	1.18	1.03	0.95	0.99	1.01	0.91	-
95% CI	4.21-9.62	1.06-1.69	0.97-1.29	1.08-1.30	0.95-1.12	0.88-1.03	0.93 1.05	0.96-1.07	0.85-0.97	-
P	<0.001	0.02	0.13	0.001	0.47	0.22	0.74	0.63	0.01	-

Non-malaria										
Hb (g/dl)	**<3**	**3-3.9**	**4-4.9**	**5-5.9**	**6-6.9**	**7-7.9**	**8-8.9**	**9-9.9**	**10-10.9**	**>=11**
Sample total	271	323	446	613	1052	1968	3282	4470	4286	4364
Cumulative n	271	594	1040	1653	2705	4673	7955	12425	16711	21075
Cumulative %	1.3	2.8	4.9	7.8	12.8	22.2	37.7	59.0	79.3	100.0
Died	22	23	40	64	126	187	223	204	150	211
% mortality	8.1	7.1	9.0	10.4	12.0	9.5	6.8	4.6	3.5	4.8
Odds ratio	1.41	1.11	1.17	1.18	1.19	1.13	1.07	1.01	0.99	-
95% CI	0.91-2.19	0.89-1.37	1.05-1.30	1.10-1.26	1.15-1.24	1.10-1.16	1.05-1.10	0.99-1.03	0.94-0.9	-
P	0.13	0.35	0.01	0.001	0.001	0.001	0.001	0.001	0.3	

Figure 3. Odds of mortality by haemoglobin (Hb) level among those with *Plasmodium falciparum* malaria (blue) and those without (red). Odds ratios compared risk of mortality at each Hb level to that above that specific threshold.

Effect of transfusion on outcome

We then investigated whether mortality risk was affected by the receipt of a transfusion during admission (Table 4a & Table 4b). In those with malaria (Table 4a), compared children who were not transfused, receipt of a transfusion appeared to improve survival for those with Hb <4g/dl but the confidence intervals were very wide (and included a possibility of harm) owing to small numbers. For all Hb levels above 4g/dl our analysis indicates that mortality was significantly higher in those receiving a transfusion compared to the non-transfused group (Table 4a).

For non-malarial cases (Table 4b), the receipt of a transfusion did not appear to result in a survival benefit irrespective of baseline haemoglobin level compared to children who were not transfused. Children with Hb ≥8g/dl had a substantially increased risk of mortality if transfused; however, this included a heterogeneous group of children in whom the underlying disease or cause of admission was an important determinant of their outcome (foot of Table 4b).

Finally, we examined whether the addition of clinical signs of severity (deep acidotic breathing and/or altered consciousness (prostration or coma)) were useful in identifying particularly high-risk groups in whom the receipt of a transfusion may be of benefit (Table 5). Overall this group had a much higher mortality, and whilst the receipt of a transfusion may have improved survival for those with a Hb of <5g/dl numbers were too small to show a statistical difference. For those with a Hb ≥5g/dl and signs of severity, transfusion was associated with a substantially increased risk of mortality (Table 5).

Discussion

We have shown in a retrospective analysis of 29,226 unselected children admitted to hospital on the coast of Kenya that the burden of moderate and profound or severe anaemia is substantial, affecting 15.8% of all admissions over 60 days of age, and that it carries a higher risk of in-hospital mortality (6.7–7.6%) compared to children with admission Hb >9g/dl (4%). The Hb level associated with the highest case fatality was different in children with malaria (all levels below Hb 3g/dl, 17.5%) in comparison to those without malaria (maximal at Hb 6-7g/dl; 12%). Receipt of a whole blood transfusion was associated with improved survival in malarial cases but only at admission Hb levels of <4g/dl, whereas transfusion in non-malaria cases did not appear to improve survival, irrespective of haemoglobin level, although causality cannot be inferred. These data are important when considering the current WHO recommended transfusion thresholds, especially for parts of sSA where malaria has declined or is of less public health consequence and where reconsideration of the current guidelines may be warranted. Whilst our data provide support for the current recommendations and indicate that it may be too soon to amend these, in non-malarious areas there are a number of notable limitations in both the evidence-base for the current guidelines and potential biases within our findings.

Overall, our findings were that at our centre compliance with WHO transfusion guidelines was good, specifically only 2.1% of children with with admission Hb >6g/dl received a transfusion. This may have been due to the availability of repeated measures of Hb post admission to monitor children. This finding contrasts for other reports where adherence to transfusion guidelines were not followed[9,10], since many transfusions initiated solely for severe pallor, a sign with poor specificity[14].

Earlier studies have relied on small samples and included little information on confounders, thus limiting the generalizability of the findings. Brabin and colleagues[11] reviewed studies reporting

Table 5. Children with signs of severity: odds of death at each level of haemoglobin (Hb) in children who received transfusion versus no transfusion.

Hb level g/dl	Total	Deaths	Not transfused	Deaths non-transfused	Case fatality	Transfused	Deaths transfused	Case fatality	Odds ratio (95% CI)	P value
<3	135	30	30	9	30.00%	105	21	20.0%	0.58 (0.23 - 1.46)	0.249
3-3.9	167	22	32	6	18.75%	135	16	11.9%	0.58 (0.21 - 1.63)	0.304
4-4.9	215	32	70	11	15.71%	145	21	14.5%	0.91 (0.41 - 2.01)	0.812
5-5.9	252	40	137	13	9.49%	115	27	23.5%	2.93 (1.43 - 5.99)	0.003
6-6.9	306	60	247	37	14.98%	59	23	39.0%	3.63 (1.93 - 6.80)	<0.001
7-7.9	410	66	377	51	13.53%	33	15	45.5%	5.33 (2.53 - 11.23)	<0.001
8-8.9	533	72	504	57	11.31%	29	15	51.7%	8.40 (3.86 - 18.31)	<0.001
9-9.9	624	71	608	63	10.36%	16	8	50.0%	8.65 (3.14 - 23.85)	<0.001
10-10.9	601	61	585	53	9.06%	16	8	50.0%	10.04 (3.62 - 27.83)	<0.001
>=11	654	93	634	83	13.09%	20	10	50.0%	6.64 (2.68 - 16.43)	<0.001
Total	3897	547	3224	383	11.9%	673	164	24.4%		

case fatality from malarious areas in sSA and found wide variations in outcome. The mean in-hospital case-fatality rate for severe anaemia (Hb <5 or <6g/dl depending on study definition) was 9% (range 4–39%). While mortality was significantly higher in children with a Hb <5g/dl (pooled RR=1.92 vs >5g/dl, 95% CI 1.7–2.2), evidence for an increased risk with less severe anaemia was not conclusive: although the risk of death was increased for a Hb <8g/dl, the confidence intervals were wide[11]. The heterogeneous group of children included and outcomes observed also make it difficult to draw specific conclusions. Our study included an unselected paediatric hospital cohort and included data on clinical severity and co-morbidities. As such the cohort represents a typical paediatric population in malaria-endemic African hospitals. A further strength of our study is that it was conducted in a setting where clinicians were largely compliant with WHO transfusion guidelines. For example, for children with a Hb >6g/dl at hospital admission only 520/24372 (2.1%) received a transfusion, and overall 67% of transfusions given were ‘’appropriate’ but this was only judged from admission criteria, the number subsequently developing severe and complicated anaemia, particularly in those with Hb 4-6g/dl admission is unknown. Overall, for children who did not meet the criteria for transfusion according to the guidelines (at admission), only 2.8% (714) received a transfusion this compares very favourably with 51% at a hospital 60 km away in Mombasa, which report over a similar time period where 51% of children who received transfusion who did not met WHO guidelines[15]. In the TRACT trial, in children with severe uncomplicated anaemia (Hb 4-6g/dl) who were not randomised to receive an immediate transfusion, close clinical and Hb monitoring for *de novo* development of signs of severity or haemoglobin drops to < 4g/dl demonstrated that 386/787 (49%) of children developed severe and complicated anaemia, 295 of these were due to drops in the Hb < 4g/dl[16].

What new knowledge this study contributes

Our current analyses indicate that for children with malaria there may be a benefit of transfusion for those with profound anaemia in terms of short-term outcome (in-hospital mortality). However, for hospital admissions without malaria the receipt of a transfusion may not be beneficial irrespective of haemoglobin levels including children with profound malarial anaemia. The major limitation of these types of analyses, whilst informative about which groups to target for further evaluation of clinical practice, the nature of the design precludes any inference of causality – which can only reliability be tested in a clinical trial. For example, children with a higher admission Hb (>8g/dl) transfusion appeared to be associated with a substantially worse outcome. Exploring the final diagnoses of this group reveals that it includes a large number of children with underlying co-morbidities associated with a substantially worse outcome including malignancies, HIV infection, severe malnutrition and trauma (see Table 2b).

In current guidelines, it is recommended that wherever possible, simple alternatives to transfusion (such as crystalloids and colloids) should be used to avert unnecessary transfusion in emergencies. However, a large paediatric controlled trial of fluid resuscitation (FEAST trial) examining boluses of 20–40mls/kg of 0.9% saline and 5% human albumin in African children with shock, including 987 (32%) with severe anaemia (Hb <5g/ld.) demonstrated a 3.3% increased absolute risk of death by 48-hours (primary outcome) in the bolus-arms compared with controls (no bolus fluid strategy)[13]. Excess mortality in bolus arms was evident in children without severe anaemia (Hb ≥5g/ld.; relative risk 1.31 (95% confidence interval 0.93-1.84)) as well as those severe anaemia (Hb <5g/ld.; RR 1.71 (1.16-2.51)) with no apparent heterogeneity between these sub-groups (p=0.31)[13]. For the conditions studied in the trial, largely malaria and sepsis, these challenge current fluid management guidelines for children with shock but are also relevant to the recommendation for use of alternatives to transfusion.

The conservative transfusion guidelines were developed to protect scarce resources, avert overuse, and reduce the risk of transfusion-transmissible infections. However, in recent years considerable progress on strengthening transfusion services, and improving the supply and safety of transfusion through establishment of regional centres to replace hospital-based systems and by providing quality assurance for viral testing[17]. Thus, the capacity of transfusion services to provide blood has greatly increased due to year-on-year declines in the intensity of malaria transmission that have led directly to reductions in hospitalisation of children with malaria, and indirectly to reduced utilisation of blood transfusion services[18]. The reduction in the burden of malaria[19], coupled with continued poor outcomes from severe anaemia irrespective of malaria incidence, are a good starting point from which to now advocate for re-evaluation of transfusion guidelines in order to generate an evidence base for clinical practice. Such evaluation is particularly pertinent given that current recommendations were designed for areas with a high proportion of malaria-associated anaemia as opposed to severe anaemia secondary to other aetiologies where mortality appears to be much higher[13,20]. We have shown previously that a reduction in the transmission intensity within Kilifi District resulted in a substantial decline in malaria and paediatric admissions to the Kilifi County Hospital[21]. Concurrent with this epidemiological transition we found a sharp decline in the prevalence of severe anaemia as well as the number and proportion of admissions transfused[18]. Nevertheless, we found no evidence that this resulted in improved outcome, which remained constant over time despite a decrease in demand for blood on the transfusion services[18].

A Cochrane review including the only two African randomised controlled trials[22,23] conducted to date (involving 114 and 116 children randomised to blood transfusion or oral haematinics) concluded that there was insufficient information on whether routinely giving blood to clinically stable children with severe anaemia either reduces death or results in a higher haematocrit measured at one month and indicated the need for a definitive trial[24]. A prospective, randomised, controlled, non-inferiority trial in relatively stable Canadian and European children demonstrated that a restrictive transfusion protocol (with a transfusion threshold <7g/dl) was as safe as a liberal protocol (threshold <9g/dl)[25]. Subsequently, practice guidelines in these countries have been amended to include restrictive transfusion (Hb<7g/dl). Worldwide consensus transfusion guidelines

for stable children on intensive care units, based on the TRIPICU trial[25], recommend transfusion at haemoglobin <7g/dl[26]. However they highlight the need for further trials, particularly in children with haemoglobins 5–7g/dl. Of note children in TRIPICU were likely to have had steady-state haemoglobins 11–14g/dl, conversely s African children living in areas where malaria is endemic and alpha + thalassaemia are common[27] typically have haemoglobin 9–11g/dl[12,28]. The apparent safety of not transfusion all children with lower haemoglobin in Africa may therefore reflect the differences in the steady-state haemoglobins.

Conclusions

Despite poor compliance with current guidelines outcomes are unsatisfactory including high rates of in-hospital mortality (9–10%) and in the six months following admission with severe anaemia both case fatality and relapse remain high (6%) thus warranting a definitive trial to establish best transfusion and treatment strategies to prevent both early and delayed mortality and relapse. The Transfusion and Treatment of severe anaemia in African children: a randomised controlled trial (ISRCTN84086586) was designed to evaluate current transfusion recommendations against more liberal transfusion to improve short term and long-term outcomes to 6 months (infection prophylaxis and multi-mineral multivitamin supplementation)[29].

Data availability

Underlying data

Figshare: Admission records, including haemoglobin measurements, for 29,226 children admitted to a paediatric ward in a rural Kenyan hospital for the period 2002–2009, https://doi.org/10.6084/m9.figshare.7635908[30]

Ethics approval, consent and permissions

The Kilifi clinical surveillance was approved by Kenyan Medical Research Institute Scientific Steering Committee and National Ethics Review Committee.

Informed, written consent was obtained from parents/guardians of the research participants prior to enrolment in the surveillance studies.

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References

1. **The prevention and management of severe anaemia in children in malaria-endemic regions of Africa: a review of research**. Geneva: World Health Organization, 2001.
2. **Global status report on blood safety and availability 2016**. Geneva: World Health Organization, 2017.
Reference Source
3. **Global Database on Blood Safety: Report 2004–5**. Geneva: World Health Organization, 2008.
Reference Source
4. Tapko J, Sam O, Diarra-Nama A: **Status of blood safety in the WHO African Region: report of the 2004 survey**. Brazzaville: WHO Regional Office for Africa;. Geneva: World Health Organization, 2007.
5. **Utilization and supply of human blood and blood products in Twenty-eighth World Health Assembly**. Geneva: World Health Organization (WHO). 1975.
Reference Source
6. **Hospital Care for Children: guidelines for the management of common illnesses with limited resources**. Geneva, Switzerland: World Health Organization, 2005.
Reference Source
7. **Pocket book of hospital care for children: Second edition Guidelines for the management of common childhood illnesses**. Geneva: World Health Organization; 2013.
Reference Source
8. Hassell O, Bates I, Maitland K: **Blood Transfusion in Resource-Limited Settings**. In: Claney N, ed. *Hunter's Tropical Medicine* 9th ed. London: Magill – Maguire – Ryan - Solomon; 2012.
Reference Source
9. Kiguli S, Maitland K, George EC, *et al.*: **Anaemia and blood transfusion in African children presenting to hospital with severe febrile illness.** *BMC Med.* 2015; **13**: 21.
PubMed Abstract | Publisher Full Text | Free Full Text
10. Opoka RO, Ssemata AS, Oyang W, *et al.*: **High rate of inappropriate blood transfusions in the management of children with severe anemia in Ugandan hospitals.** *BMC Health Serv Res.* 2018; **18**(1): 566.
PubMed Abstract | Publisher Full Text | Free Full Text
11. Brabin BJ, Premji Z, Verhoeff F: **An analysis of anemia and child mortality.** *J Nutr.* 2001; **131**(2S–2): 636S–645S; discussion 646S-648S.
PubMed Abstract | Publisher Full Text
12. Phiri KS, Calis JC, Faragher B, *et al.*: **Long term outcome of severe anaemia in Malawian children.** *PLoS One.* 2008; **3**(8): e2903.
PubMed Abstract | Publisher Full Text | Free Full Text
13. Maitland K, Kiguli S, Opoka RO, *et al.*: **Mortality after fluid bolus in African children with severe infection.** *N Engl J Med.* 2011; **364**(26): 2483–95.
PubMed Abstract | Publisher Full Text
14. Olupot-Olupot P, Prevatt N, Engoru C, *et al.*: **Evaluation of the diagnostic accuracy and cost of different methods for the assessment of severe anaemia in hospitalised children in Eastern Uganda [version 1; referees: 1 approved].** *Wellcome Open Res.* 2018; **3**: 130.
Publisher Full Text
15. Nabwera HM, Fegan G, Shavadia J, *et al.*: **Pediatric blood transfusion practices at a regional referral hospital in Kenya.** *Transfusion.* 2016; **56**(11): 2732–2738.
PubMed Abstract | Publisher Full Text | Free Full Text
16. Maitland K, Kiguli S, Olupot-Olupot P, *et al.*: **Immediate Transfusion in African Children with Uncomplicated Severe Anemia.** *N Engl J Med.* 2019; **381**(5): 407–19.
PubMed Abstract | Publisher Full Text

17. Centers for Disease Control and Prevention (CDC): **Progress toward strengthening blood transfusion services--14 countries, 2003-2007.** *MMWR Morb Mortal Wkly Rep.* 2008; **57**(47): 1273–7.
PubMed Abstract

18. Pedro R, Akech S, Fegan G, *et al.*: **Changing trends in blood transfusion in children and neonates admitted in Kilifi District Hospital, Kenya.** *Malar J.* 2010; **9**: 307.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Njuguna P, Maitland K, Nyaguara A, *et al.*: **Observational study: 27 years of severe malaria surveillance in Kilifi, Kenya.** *BMC Med.* 2019; **17**(1): 124.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Nadjm B, Amos B, Mtove G, *et al.*: **WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study.** *BMJ.* 2010; **340**: c1350.
PubMed Abstract | Publisher Full Text | Free Full Text

21. O'Meara WP, Bejon P, Mwangi TW, *et al.*: **Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya.** *Lancet.* 2008; **372**(9649): 1555–62.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Holzer BR, Egger M, Teuscher T, *et al.*: **Childhood anemia in Africa: to transfuse or not transfuse?** *Acta Trop.* 1993; **55**(1–2): 47–51.
PubMed Abstract | Publisher Full Text

23. Bojang KA, Palmer A, Boele van Hensbroek M, *et al.*: **Management of severe malarial anaemia in Gambian children.** *Trans R Soc Trop Med Hyg.* 1997; **91**(5): 557–61.
PubMed Abstract | Publisher Full Text

24. Meremikwu M, Smith HJ: **Blood transfusion for treating malarial anaemia.** *Cochrane Database Syst Rev.* 2000; (2): CD001475.
PubMed Abstract | Publisher Full Text

25. Lacroix J, Hébert PC, Hutchison JS, *et al.*: **Transfusion strategies for patients in pediatric intensive care units.** *N Engl J Med.* 2007; **356**(16): 1609–19.
PubMed Abstract | Publisher Full Text

26. Doctor A, Cholette JM, Remy KE, *et al.*: **Recommendations on RBC Transfusion in General Critically Ill Children Based on Hemoglobin and/or Physiologic Thresholds From the Pediatric Critical Care Transfusion and Anemia Expertise Initiative.** *Pediatr Crit Care Med.* 2018; **19**(9S Suppl 1): S98–S113.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Wambua S, Mwacharo J, Uyoga S, *et al.*: **Co-inheritance of alpha+-thalassaemia and sickle trait results in specific effects on haematological parameters.** *Br J Haematol.* 2006; **133**(2): 206–9.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Staedke SG, Maiteki-Sebuguzi C, DiLiberto DD, *et al.*: **The Impact of an Intervention to Improve Malaria Care in Public Health Centers on Health Indicators of Children in Tororo, Uganda (PRIME): A Cluster-Randomized Trial.** *Am J Trop Med Hyg.* 2016; **95**(2): 358–67.
PubMed Abstract | Publisher Full Text | Free Full Text

29. Mpoya A, Kiguli S, Olupot-Olupot P, *et al.*: **Transfusion and Treatment of severe anaemia in African children (TRACT): a study protocol for a randomised controlled trial.** *Trials.* 2015; **16**(1): 593.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Maitland K, Ohuma EO, Mpoya A: **Admission records, including haemoglobin measurements, for 29,226 children admitted to a paediatric ward in a rural Kenyan hospital for the period 2002-2009.** *figshare.* Fileset. 2019.
http://www.doi.org/10.6084/m9.figshare.7635908

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 04 October 2019

https://doi.org/10.21956/wellcomeopenres.16831.r36188

✔ **Hans Ackerman**
Laboratory of Malaria and Vector Research, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

Major Comments -
"Our current analyses indicate that for children with malaria there may be a benefit of transfusion for those with profound anaemia in terms of short-term outcome (in-hospital mortality). However, for hospital admissions without malaria the receipt of a transfusion may not be beneficial irrespective of haemoglobin levels including children with profound malarial anaemia."

These statements appear contradictory regarding the benefit (or not) of transfusion among children with malaria and profound anemia. Perhaps the last part of the sentence ("...including children with profound malarial anaemia.") was meant to be removed?

Minor Comments -
"Overall, our findings were that at our centre compliance with WHO transfusion guidelines was good, specifically only 2.1% of children with with admission Hb >6g/dl received a transfusion."

It may be worth balancing that statement with a comment about poor adherence among those with Hb 4-6.

Typos, etc. - in brackets, in bold
"In those with malaria (Table 4a), compared **[to]** children who were not transfused, receipt of a transfusion appeared to improve survival for those with Hb <4g/dl but the confidence intervals were very wide (and included a possibility of harm) owing to small numbers."

"For example, **[among]** children with a higher admission Hb (>8g/dl) **[,]** transfusion appeared to be associated with a substantially worse outcome."

"However, a large paediatric controlled trial of fluid resuscitation (FEAST trial) examining boluses of 20–40mls/kg of 0.9% saline and 5% human albumin in African children with shock, including 987 (32%) with severe anaemia (Hb <**5g/ld**.) demonstrated a 3.3% increased absolute risk of death by 48-hours

(primary outcome) in the bolus-arms compared with controls (no bolus fluid strategy)[13]. Excess mortality in bolus arms was evident in children without severe anaemia (**Hb ≥5g/ld**.; relative risk 1.31 (95% confidence interval 0.93-1.84)) as well as those severe anaemia (**Hb <5g/ld**.; RR 1.71 (1.16-2.51)) with no apparent heterogeneity between these sub-groups (p=0.31)[13]."

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Critical Care Medicine, Vascular Biology, Hematology and Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 August 2019

https://doi.org/10.21956/wellcomeopenres.16831.r36187

Rose McGready
Shoklo Malaria Research Unit, Mahidol University, Mae Sot, Thailand

I have reviewed Version 2 and the response to comments have been satisfactorily addressed by the authors. The inclusion of the TRACT manuscripts provided in this version was worth waiting for. This is very important to get indexed and provides good evidence to support a clinical trial on transfusion in children not just for sSA but other parts of the globe e.g. South and South East Asia where severe *P. falciparum* occurs.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malaria pregnancy, severe malaria, complicated malaria, drug resistant malaria, guidelines for management of malaria and anaemia in resource limited settings

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 06 March 2019

https://doi.org/10.21956/wellcomeopenres.16367.r34837

Rose McGready
Shoklo Malaria Research Unit, Mahidol University, Mae Sot, Thailand

The authors have presented a significant data bundle (8 years, >29,000 children) to understand survival in transfused children when using the thresholds in the WHO guidelines. Other admission characteristics or investigations e.g. malaria, signs of severity; were included in the analysis. The very high burden and mortality from profound and severe anaemia, 10 years ago (2002-2009) in Kilifi Kenya, was presented. The manuscript is well written and a large amount of information (a great deal of clinical work) has been packaged together to provide insight on an important subject.

Major:

1. The data is nearly 10 years old – can the reason for presenting this particular 2002-2009 cohort be explained?
2. Is there overlap between FEAST study and this large dataset? If yes – that is a bit different to routine hospital admission and treatment.
3. The take away message (as in first sentence of the abstract - conclusion, that states that transfusion does not improve outcome in severe and complicated anaemia) is profound, and contrary to long held belief/practices. Are there important missing data that are unavailable or is there a limitation to the data analysis? This bold statement comes from a stratified analysis. There is sometimes a frustrating amount of missing data e.g. base excess, HIV in Table 3. Is it the missing data that precluded multivariate analysis? Can all/more variables be added to the Table 5 analysis? Does mortality risk change over time (year of admission)?

Minor:

1. Affiliation 2 has two commas after facility.
2. Abstract Background: These first 3 sentences lack cohesion. For the first sentence – not just pediatric transfusion that are challenging ….all transfusion? What is the connection between restrictive transfusion practices (from HIC?) and the next statement about survival, malaria +/- transfusion.
3. Abstract Methods: A retrospective analysis of hospital records of children? Digital records? Paper records?
4. sSA undefined at first use in Conclusion of Abstract.
5. Introduction - Wording problem in this sentence: For example, the 2016 WHO survey found that of 46 countries reporting in the WHO African Region, which are home to approximately 13% of the global population collected a total of about 5.6 million blood donations and accounted for only about 4% of global donations[2].
6. What is HDI?
7. Methods 2nd paragraph - Wording problem: "…systematically collected on all admission…
8. Discussion – section what new knowledge this study contributes.
9. The later half of the 2nd sentence "…receipt of a transfusion MAY NOT be beneficial irrespective of haemoglobin levels including children with PROFOUND malarial anaemia" appears contradictory to the first ½ of the first sentence of the same paragraph "…for children with malaria there MAY be a benefit of transfusion for those with PROFOUND anaemia".
10. It would be useful for the reader to include the sample size of the Canadian and European restrictive transfusion trial (given the sample size data at the start of the paragraph).
11. Malaria has decreased but has HIV has as well?
12. Conclusion, 2nd word: 'poor' or 'good' compliance with current guidelines?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: I have been a collaborator on one paper with one of the authors EO Ohuma, published in 2017. doi: 10.1002/uog.17347

Reviewer Expertise: Malaria pregnacy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2019

Kathryn Maitland, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

Major:
The data is nearly 10 years old – can the reason for presenting this particular 2002-2009 cohort be explained? Is there overlap between FEAST study and this large dataset? If yes – that is a bit different to routine hospital admission and treatment.

Yes the data sets were assembled to inform the design of the TRACT trial (and justify to funders) - see reference to trial protocol. The data were collected during a period when malaria transmission in Kilifi had transitioned from high endemicity to low (its nadir actually), based upon the recent paper published about severe malaria (ref 1 below)**. FEAST trial only started in 2009 and the numbers included in the trial from Kilifi were relatively small and I do not think this would have influenced the overall findings.**

The take away message (as in first sentence of the abstract - conclusion, that states that transfusion does not improve outcome in severe and complicated anaemia) is profound, and contrary to long held belief/practices. Are there important missing data that are unavailable or is there a limitation to the data analysis? This bold statement comes from a stratified analysis. There is sometimes a frustrating amount of missing data e.g. base excess, HIV in Table 3. Is it the missing data that precluded multivariate analysis? Can all/more variables be added to the Table 5 analysis? Does mortality risk change over time (year of admission)?

We have moderated the sentence to say severe anaemia. The only parameters that were collected routinely were clinical data, haemoglobin, malaria slide and blood cultures. HIV was only recorded on patients following parental assent; thus missingness in this data field is likely to be bias the overall interpretation. Blood gases were not routinely collected and only done in children with signs of severity and at a clinicians discretion.

Minor:
Affiliation 2 has two commas after facility.

We have corrected these.

Abstract Background: These first 3 sentences lack cohesion. For the first sentence – not just pediatric transfusion that are challenging ….all transfusion? What is the connection between restrictive transfusion practices (from HIC?) and the next statement about survival, malaria +/- transfusion.
Abstract Methods: A retrospective analysis of hospital records of children? Digital records? Paper records? sSA undefined at first use in Conclusion of Abstract.

We have corrected these and rewritten the first 3 sentences to make our points clearer. The methods of data collection are made clearer.

Introduction - Wording problem in this sentence: For example, the 2016 WHO survey found that of 46 countries reporting in the WHO African Region, which are home to approximately 13% of the global population collected a total of about 5.6 million blood donations and accounted for only about 4% of global donations. What is HDI? Methods 2 paragraph - Wording problem: "…systematically collected on all admission…

We have edited these.

Discussion – section what new knowledge this study contributes.

We have included this.

The latter half of the 2 sentence "…receipt of a transfusion MAY NOT be beneficial irrespective of haemoglobin levels including children with PROFOUND malarial anaemia" appears contradictory to the first ½ of the first sentence of the same paragraph "…for children with malaria there MAY be a benefit of transfusion for those with PROFOUND anaemia".

Sorry, thank you for pointing out this important typo - we agree that the later sentence is the correct version.

It would be useful for the reader to include the sample size of the Canadian and European restrictive transfusion trial (given the sample size data at the start of the paragraph).

We are not sure what a sample size would add for observational data? The Canadian/European cohorts were much lower sample sizes and based on non-inferiority.

Malaria has decreased but has HIV has as well?

Conclusion, 2 word: ‘poor’ or ‘good’ compliance with current guidelines?

We don't have good evidence that there have been changes in HIV overtime; we do however for malaria.
We have clarified wording in the conclusions.

1. Njuguna P, Maitland K, Nyaguara A, et al. Observational study: 27 years of severe malaria surveillance in Kilifi, Kenya. *BMC Med* 2019; **17**(1): 124.

Competing Interests: No competing interests were disclosed.

Reviewer Report 06 March 2019

https://doi.org/10.21956/wellcomeopenres.16367.r34854

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Hans Ackerman
Laboratory of Malaria and Vector Research, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

Summary: This is a prospective observational study of consecutive admissions of children age 60 days to 15 years to Kilifi County Hospital during the years 2002-2009. The primary goal of the analysis was to calculate in-hospital mortality within each hemoglobin stratum (e.g., 3.0-3.9 g/dL). Secondary goals were to see if mortality by Hb stratum differed between children with malaria versus children hospitalized for other reasons, and to see if mortality was different between those who received a blood transfusion versus those who did not.

Strengths of this study include: (1) established data collection practices; (2) routine clinical evaluations including physical assessment, complete blood count, blood culture, HIV testing, and blood film for malaria parasites; (3) consistent method of determining Hb concentration (Coulter counter); and (4) a large sample size with 29,226 included in the analysis. With this wealth of data from a single site over many years, there is the potential to learn more about transfusion practice and transfusion thresholds.

However, the use of stratification instead of adjustment (e.g., multivariable regression modeling) limits the conclusions that can be drawn from the data. The results could look different if adjustment for disease severity were applied instead of the current approach of stratification and subgroup analysis. Take, for example, the analysis presented in Table 4a. This table shows the case fatality rate for children with malaria stratified by hemoglobin level, comparing those who were transfused against those who were not transfused. When the Hb level was in the range of 5.0-5.9 g/dL, the mortality in the not transfused group was 16/479 (3.3%) and in the transfused group it was 20/178 (11.2%), giving an unadjusted odds ratio for death associated with transfusion of 3.66 (95%CI 1.85-7.24, $p < 0.001$) - in other words, transfusion appears to be associated with a substantially increased odds of death in children with malaria who have a hemoglobin in the range of 5.0-5.9 g/dL.

The problem with this analysis is that it doesn't consider all the information available for each patient. We know that WHO guidelines were followed most of the time at Kilifi County Hospital, and that to transfuse a child with malaria whose hemoglobin is in the range of 4.0 - 6.0 g/dL, an additional life-threatening complication would need to be present to justify the transfusion. These life-threatening complications could include any one of the following: shock, severe dehydration, impaired consciousness, respiratory distress or high parasitemia. The presence of these life-threatening complications in the transfused group (and to a much lesser extent in the not transfused group) could explain the higher mortality observed. To accurately determine the association between transfusion and death, one needs to account for the underlying differences in disease severity that may exist between the transfused and not transfused groups. In addition to the factors listed above, one may need to include base excess, age, nutritional status and possibly other factors that affect the outcome of hospitalization in a multivariable analysis.

The authors have approached this issue with a subgroup analysis shown in Table 5 where the analysis was limited only to those children who have deep acidotic breathing and/or altered consciousness. While it would appear that transfusion is largely futile, and possibly harmful, in this subgroup of severely ill children, it is hard to interpret the results when other potential determinants of outcome such as age, malaria infection, HIV status, nutritional status, trauma, etc., might not be equally distributed across the transfused and not transfused groups like they would be if this were a randomized controlled trial.

For these reasons, I would caution the authors not to draw conclusions from the comparisons of crude mortality rates in unadjusted analyses when the underlying disease severity may be very different among those who received transfusions compared to those who did not. I would recommend a statistical approach where the effects of multiple factors and their interactions could be accounted for.

Overall, the article represents a tremendous data collection effort that has produced a rich dataset with the potential to inform both current practice in Eastern Kenya as well as the design of randomized controlled trials for blood transfusion of children living in sub-Saharan Africa.
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Other comments:

While there appeared to be very tight adherence to WHO guidelines for Hb < 4 g/dL (912/1134 [80.4%] transfused) and for Hb > 6 (520/24,372 [2.1%] transfused), there was much less adherence to the guidelines when Hb was in the 4-6 g/dL range. In the 4-6 g/dL range, for those who met severity criteria for transfusion, only 567/1564 (36.3%) were transfused, and for those who did not meet severity criteria, 194/746 (26.0%) were still transfused - why do you think adherence was less strict when the hemoglobin was in the 4-6 g/dL range?

In the discussion, it may be worth emphasizing that while Canadian and European trials in critically ill children have established that a transfusion threshold of 7 g/dL is not worse than a transfusion threshold of 9 g/dL, this is still well above the threshold of 4 (or 6) g/dL recommended worldwide where little trial data is available.

In reference to the FEAST trial, the fluid bolus volume was up to 60 mL/kg in stratum B of that study (not 40 mL/kg as cited here).

Regarding baseline characteristics:

- Does mid-upper arm circumference need to be normalized to age?
- Was tachycardia defined using age-specific ranges?
- Was temperature gradient determined by touch or with a thermometer?

- How was coma defined?

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Minor comments:

Figure 2 would benefit from an legend explaining the comparisons being made: each Hb stratum vs the sum of all strata above that one.

Table 2b was referred to but not present in the pdf version of the article.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: Although I do not have any financial conflicts of interest, I do have a related manuscript under review at a different journal titled: "The Association of Blood Transfusion with Outcome among Africa Children Hospitalized with Plasmodium falciparum malaria: a prospective observational study".

Reviewer Expertise: Critical Care Medicine, Vascular Biology, Hematology and Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2019

Kathryn Maitland, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

Thank you for your very detailed consideration of this manuscript. First, I want to apologise for my late response to their questions, I have been unusually distracted by other commitments but also timed my response so I could refer to the TRACT trial transfusion manuscripts which are now published and help to address some of the questions raised. I will respond to the reviewers individual comments.

Summary: This is a prospective observational study of consecutive admissions of children age 60 days to 15 years to Kilifi County Hospital during the years 2002-2009. The primary goal of the analysis was to calculate in-hospital mortality within each hemoglobin stratum (e.g., 3.0-3.9 g/dL). Secondary goals were to see if mortality by Hb stratum differed between children with malaria versus children hospitalized for other reasons, and to see if mortality was different between those who received a blood transfusion versus those who did not.

Strengths of this study include: (1) established data collection practices; (2) routine clinical evaluations including physical assessment, complete blood count, blood culture, HIV testing, and blood film for malaria parasites; (3) consistent method of determining Hb concentration (Coulter counter); and (4) a large sample size with 29,226 included in the analysis. With this wealth of data from a single site over many years, there is the potential to learn more about transfusion practice and transfusion thresholds.

However, the use of stratification instead of adjustment (e.g., multivariable regression modeling) limits the conclusions that can be drawn from the data. The results could look different if adjustment for disease severity were applied instead of the current approach of stratification and subgroup analysis. Take, for example, the analysis presented in Table 4a. This table shows the case fatality rate for children with malaria stratified by hemoglobin level, comparing those who were transfused against those who were not transfused. When the Hb level was in the range of 5.0-5.9 g/dL, the mortality in the not transfused group was 16/479 (3.3%) and in the transfused group it was 20/178 (11.2%), giving an unadjusted odds ratio for death associated with transfusion of 3.66 (95%CI 1.85-7.24, $p < 0.001$) - in other words, transfusion appears to be associated with a substantially increased odds of death in children with malaria who have a hemoglobin in the range of 5.0-5.9 g/dL.

The problem with this analysis is that it doesn't consider all the information available for each patient. We know that WHO guidelines were followed most of the time at Kilifi County Hospital, and that to transfuse a child with malaria whose hemoglobin is in the range of 4.0 - 6.0 g/dL, an additional life-threatening complication would need to be present to justify the transfusion. These life-threatening complications could include any one of the following: shock, severe dehydration, impaired consciousness, respiratory distress or high parasitemia. The presence of these life-threatening complications in the transfused group (and to a much lesser extent in the not transfused group) could explain the higher mortality observed. To accurately determine the association between transfusion and death, one needs to account for the underlying differences in disease severity that may exist between the transfused and not transfused groups. In addition to the factors listed above, one may need to include base excess, age, nutritional status and possibly other factors that affect the outcome of hospitalization in a multivariable analysis.

The authors have approached this issue with a subgroup analysis shown in Table 5 where the analysis was limited only to those children who have deep acidotic breathing and/or altered consciousness. While it would appear that transfusion is largely futile, and possibly harmful, in this subgroup of severely ill children, it is hard to interpret the results when other potential determinants

of outcome such as age, children, it is hard to interpret the results when other potential determinants of outcome such as age, malaria infection, HIV status, nutritional status, trauma, etc., might not be equally distributed across the transfused and not transfused groups like they would be if this were a randomized controlled trial.

For these reasons, I would caution the authors not to draw conclusions from the comparisons of crude mortality rates in unadjusted analyses when the underlying disease severity may be very different among those who received transfusions compared to those who did not. I would recommend a statistical approach where the effects of multiple factors and their interactions could be accounted for.

Overall, the article represents a tremendous data collection effort that has produced a rich dataset with the potential to inform both current practice in Eastern Kenya as well as the design of randomized controlled trials for blood transfusion of children living in sub-Saharan Africa.

Response:
We agree that the looking at a Hb range over 1 gram increments is rather crude but the numbers in each of the levels of Hb are large. We were limited in adding in too many other variables to understand why the transfusion vs no transfusion may have had different outcomes as the only parameters that were collected routinely were clinical data, haemoglobin, malaria slide and blood cultures. HIV was only recorded on patients following parental assent; thus missingness in this data field is likely to bias the overall interpretation as those refusing the test have been shown to have worse outcome. Blood gases were not routinely collected and only done in children with signs of severity and at a clinicians discretion, thus likely to bias the interpretation if this were to be included in a multivariate analysis. Moreover, the decision to transfuse, especially in those with higher haemoglobin were not routinely collected and thus a major limitation of this paper. The only way to satisfactorily address whether children benifit from transfusion is through a clinical trial which has been conducted in those with haemoglobins below 6 g/dl (2). For those with Hb above this level this would require a separate trial, which may be harder to justify given the substantial burden that severe anaemia places on the transfusion services in Africa.

Other comments:
While there appeared to be very tight adherence to WHO guidelines for Hb < 4 g/dL (912/1134 [80.4%] transfused) and for Hb > 6 (520/24,372 [2.1%] transfused), there was much less adherence to the guidelines when Hb was in the 4-6 g/dL range. In the 4-6 g/dL range, for those who met severity criteria for transfusion, only 567/1564 (36.3%) were transfused, and for those who did not meet severity criteria, 194/746 (26.0%) were still transfused - why do you think adherence was less strict when the hemoglobin was in the 4-6 g/dL range?

Response:
Yes, we were surprised by the poor compliance in those with Hb 4-6g/dl with current guidelines especially those with signs of severity. Some may have been explained by blood shortages and by the time blood became available either the child had died or recovered- we did not record whether a transfusion was requested and not received- something for future implementation post-TRACT trial. In the TRACT trial we did note that 49% of children with Hb 4-6 g/dl without signs of severity developed severe and complicated anaemia later in the admission requiring blood transfusion(3)**. We have now**

included this into the discussion section of the paper.

In the discussion, it may be worth emphasizing that while Canadian and European trials in critically ill children have established that a transfusion threshold of 7 g/dL is not worse than a transfusion threshold of 9 g/dL, this is still well above the threshold of 4 (or 6) g/dL recommended worldwide where little trial data is available.

We have added in some more discussion about this include a review that was done regarding this.

In reference to the FEAST trial, the fluid bolus volume was up to 60 mL/kg in stratum B of that study (no 40 mL/kg as cited here).

In the controlled trial (Albumin vs Saline (Bolus) vs Control which is the reference I am referring to only received 20-40 mls/kg only (with 40 mls/kg given only in the last 6 months of the trial, after a protocol amendment) In the stratum with hypotensive shock only few children were enrolled (29 in total) and there was no controls in this stratum and in reality following the protocol amendment which permitted up 60mls/kg very few children actually received 60mls/kg.

Regarding baseline characteristics: Does mid-upper arm circumference need to be normalized to age?

This is a standard way of assessing MUAC; zscores are unusual.

Was tachycardia defined using age-specific ranges? Was temperature gradient determined by touch or with a thermometer? How was coma defined?

Yes, we have included in the methods and for tachycardia I have reference the FEAST trial.

Minor comments:
Figure 2 would benefit from a legend explaining the comparisons being made: each Hb stratum vs the sum of all strata above that one. Table 2b was referred to but not present in the pdf version of the article.

Sorry this was in error it was meant to indicate the legend below Table 4b.

1. Njuguna P, Maitland K, Nyaguara A, et al. Observational study: 27 years of severe malaria surveillance in Kilifi, Kenya. *BMC Med* 2019; **17**(1): 124.
2. Mpoya A, Kiguli S, Olupot-Olupot P, et al. Transfusion and Treatment of severe anaemia in African children (TRACT): a study protocol for a randomised controlled trial. *Trials* 2015; **16**(1): 593.
3. Maitland K, Kiguli S, Olupot-Olupot P, et al. Immediate Transfusion in African Children with Uncomplicated Severe Anemia. *The New England journal of medicine* 2019; **381**(5): 407-19.

Competing Interests: No competing interests were disclosed.