The impact of venepuncture training on the reduction of pre-analytical blood sample haemolysis rates: a systematic review

Abstract

Background: Venepuncture involves the introduction of a needle into a vein to collect a representative blood sample for laboratory testing. In the pre-analytical phase, haemolysis (the rupturing of erythrocytes and release of their contents into the extracellular compartment) has safety, quality and cost implications. Training in correct venepuncture practice has the potential to reduce *in vitro* haemolysis rates, but the evidence for this notion has yet to be synthesised.

Design: Systematic review.

Method: Published studies on the effectiveness of venepuncture training on haemolysis rates were searched in relevant databases. The McMaster critical appraisal tool was used to assess methodological quality. The GRADE tool was used to evaluate the body of evidence in relation to the research questions. Implementation Fidelity was also scrutinised in each study.

Results: Eight out of 437 retrieved studies met the inclusion criteria. None were randomised controlled trials (RCT). Between-study heterogeneity in design, intervention characteristics and the biochemical threshold for haemolysis precluded a meta-analysis. Post-training reductions in haemolysis rates of between 0.4-19.8% were reported in four of the studies, which developed their intervention according to a clear evidence-base and included mentoring in the intervention. Rises in haemolysis rates of between 1.3-1.9% were reported in two studies, while the intervention effect was inconsistent within two other studies.

Conclusion: There are no RCTS on the effectiveness of venepuncture training for reducing haemolysis rates, and findings from the existing uncontrolled studies are unclear. For a more robust evidence base, we recommend more RCTs with standardisation of haemolysis thresholds and training-related factors.

Relevance to clinical practice: While venepuncture training is an important factor influencing quality of blood sample in clinical practice, more robust evidence is needed to make specific recommendations about training content for reduction of haemolysis rates. Standardisation of haemolysis thresholds would also enable future meta-analyses.

Keywords: Pre-analytical, Venepuncture, Training, Haemolysis, Quality

What does this paper contribute to the wider global clinical community?

- Our paper highlights the absence of robust evidence that training effectively reduces haemolysis rates in clinical settings.
- We suggest that there should be global standardisation in haemolysis definitions and more RCTs on the effectiveness of clearly-defining training for reducing haemolysis rates.

Introduction

Venepuncture is a commonly-conducted procedure in the pre-analytical phase of the blood sampling process. A needle is used to obtain a representative blood sample for laboratory testing (Lavery and Ingram, 2005). Laboratory-based analyses of blood samples facilitate an estimated 60-70% of all clinical decisions on patient admissions, discharges and medication (Yazar et al 2016). Therefore, accurate and rapid collection

and reporting of analysis results is desirable. In European countries, clinicians in many different types of roles undertake venepunctures, alongside dedicated phlebotomists (Simundic et al, 2015; Makhumula-Nkhoma et al, 2015), and this obviously smooths patient flow through a clinical pathway. Nevertheless, the involvement of many clinicians in the venepuncture service may increase the variability of quality in the venepuncture practice and, therefore, quality of the blood sample for subsequent analysis. One important indicator of blood sample quality is the rate (proportion of all blood samples over a defined time period) of pre-analytical blood sample haemolysis.

When a blood sample is "haemolysed", there are damaged and disrupted erythrocytes; leading to the release of haemoglobin and other intracellular components from inside the cell, to the surrounding plasma (Lippi et al, 2008). Haemolysis directly affects sample integrity by increasing or decreasing the concentration gradient between the cells and plasma. Released constituents interfere with chemical reactions used during the sample analysis. Poor sample quality due to haemolysis leads to sample re-collection, delayed turn-around-time and has cost bearing on the patients, staff and organisation. Blood sample haemolysis has been reported to put patients at risk and impacts on the safety of both patients and staff as well as potentially affecting the quality of care (Yazar et al, 2016; Bolenius et al, 2013; Lillo et al, 2012; Ong et al, 2009).

Haemolysis may occur *in vivo* caused by unpreventable biological influences, leading to inherently high concentration of potassium in a particular patient; and/or *in vitro* due to preventable interference factors such as incorrect drawing and preparation of the sample (Kirschbaumweg, 2002). *In vitro* haemolysis can be influenced by different

types of equipment, e.g. cannula vs needle venepuncture (Heyer et al, 2012), different collection sites, e.g. how distal to the antecubital fossa the sample is obtained (Heyer et al, 2012) and variability in protocol, e.g. whether the tourniquet is left on for more than a minute during venepuncture or not (Reed et al, 2016; Saleem et al, 2009). There are also inter-clinic differences in transportation and storage of blood samples, e.g. using a pneumatic transportation tube vs delivery by hand (McCaughey et al, 2016); or variation in storage temperature (Romero et al, 2017). *In vitro* haemolysis is reported to account for 39-69% of all unsuitable samples received in the pre-analytical phase (Lippi et al, 2008; Lippi et al, 2012). The International Federation of Clinical Chemistry model of Quality Indicators defines this type of haemolysis as a free haemoglobin (fHb) concentration >0.5 g/L (IFCC, 2017). Our review is based on *in vitro* blood sample haemolysis.

It is the priority of every professional and health care organisation to provide safe, quality and timely care to the patients (HEE, 2016). Likewise, this is the expectation of every patient. Any delays in the provision of care due, for example, to haemolysed blood samples, can prolong hospital stay and increase costs (Ong et al, 2009). As *in vitro* blood sample haemolysis is preventable, appropriately designed and standardised processes could facilitate the reduction in haemolysis rate (Yazar et al, 2016). Nevertheless, for the proper handling of the equipment and successful implementation of processes, the operator requires good education and training in the conduct of venepuncture and in haemolysis prevention. There are different approaches to venepuncture education and training, including the content, intensity and moderation with varying quality (Bolenius et al, 2013; Cadamuro et al, 2016; Lillo et al, 2012; Cockill, 2012; Ong et al, 2009; Romero et al, 2012, Romero et al, 2017 and Yazar et al, 2016).

The inherent complexity in venepuncture education and training encompasses involvement of different professions, and the availability of the staff to attend the training from the already busy clinical areas. Venepuncture training could, therefore, be considered a complex intervention (Moore et al, 2015). The fidelity of the training could equally be variable. Health Education England (HEE) highlighted the lack of sound scientific evidence on the most effective types of education and training to improve patient outcomes and safety (HEE, 2016). Similarly, there is lack in evidence based venepuncture education and training with positive impact in haemolysis rate reduction.

Aim

The aim of this research was to synthesise the evidence for the effectiveness of training for reducing pre-analytical blood sample haemolysis rates. We pose the following questions:

1) What impact does venepuncture training have on the haemolysis rates?

2) How sustainable are any beneficial effects of the training on the reduction of Haemolysis rates?

3) Are there any specific characteristics of training programmes that are particularly effective?

Methods

A systematic review (supplementary file 1) is reported using the Reporting Outcomes for Systematic Reviews and Meta-analysis (PRISMA) statement (Moher et al, 2009). Three reviewers (NN, RM and GA) developed the inclusion and exclusion criteria.

Studies conducted in any areas or departments in the primary, secondary and tertiary services, in any geographical region of the world, describing the effect of venepuncture training on haemolysis rate, were considered for the review. Only those studies published in English were included in the review. No publication date limits were set in the various databases.

Randomised controlled trials (RCTs), uncontrolled single arm studies and single-or multi-centre studies were considered for inclusion in the review. If the study was a RCT, the details of comparator were recorded. Uncontrolled single arm pre / post designed studies were also included. Studies on the effects of complex interventions that were multifaceted and where training was not the primary component of the intervention were excluded. Conference abstracts were excluded as study quality could not be appraised.

Search strategy

A comprehensive electronic search of literature was performed by NN in; British Nursing Index (BNI) (1992 to 2018), Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1981 to 2018), Excerpta Medica (EMBASE) (1974 to 2018), Medline (1946 to 2018), PubMed (1946 to 2018) and Trial Protocols through Clinical Trial.gov website. Both British and American spellings and different forms of the search words which appeared in prior reviewed literature were catered for in the search. The primary terms medical subject heading (MeSH) and keywords were used to facilitate retrieval of relevant literature. Thesaurus facilities were used for some of the search terms for example, venepuncture/venipuncture/phlebotomy and either a

‘major’ or ‘explode’ or both were used depending on the database functionality (table 1).

Table 1: Primary search terms and strategy

Elements	**Search terms**
Population	venepuncture *OR* ‘’vene puncture’’ *OR* ‘’veni puncture’’ *OR* venepuncture *OR* phlebotomy *OR* ‘’blood sampling’’ *OR* ‘’blood sample collection’’ *AND* 
Intervention	training *OR* initiative *OR* intervention *OR* program* *AND* 
Comparator	No training *AND* 
Outcome	haemolys* *OR* hemolys* *OR* hemolyz* *OR* preanalytical *OR* ‘’pre-analystical’’

The search was performed twice; in December, 2016 with an update search in August 2018 to enable inclusion of all relevant new research before data extraction. Duplicate citations were excluded using the software, Refworks. Manual searches were conducted through reference lists of all eligible studies. Study authors were contacted for clarification of data in some of the studies.

Selection of studies and data extraction

Our review reporting approach was based on the Preferred Reporting Outcomes for Systematic Reviews and Meta-analysis (PRISMA) statement (Moher et al, 2009). Processing in the conduct followed a publically available pre-registered protocol; ID: 42017059658 (https://www.crd.york.ac.uk/PROSPERO/myprospero.php). This maximised transparency, targeted search and prevented bias and unnecessary duplication of research question. Title and abstract screen of retrieved literature was conducted by NN and RM independently. The selected lists were compared and differences discussed. GA was consulted when agreement was not reached; this reduced subjectivity and potential bias in the study selection process.

Data analysis

The following data were extracted from each included study and summarised under: 1) country research was conducted, 2) study setting and participants, 3) design, 4) haemolysis rate threshold, 5) quality assessment and 6) implementation fidelity (IF).

There was heterogeneity in the study designs, the intervention including time spent for the intervention and, especially, in the method of haemolysis detection. In agreement, McCaughey et al. (2016) reported that both automated and visual methods are used in the various studies for detecting haemolysis. Automated detection of haemolysed samples are pre-set by the manufacturers of the analysers and are based on the analyte being analysed (Goyal and Schortzer, 2015). This heterogeneity precluded a meta-analysis of effect sizes in our systematic review. There were also differences in reporting haemolysis index (HI) with some reporting fHb in milligrams per decilitre (mg/dL) and others in grams per Litre (g/L).

Quality assessment

Methodological quality was conducted using the McMaster critical appraisal tool for the assessment of quantitative studies methodological quality and risk of bias, consistency and generalizability (Law et al. 1998). Its selection was based on the level of detail it allows and its relevancy to the heterogeneous study designs that were selected by the various study authors. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) Framework was used to evaluate the body of evidence in relation to the research questions. This gave a summary across different studies under: 1) methodological limitations of the studies, 2) indirectness, 3) imprecision, 4) inconsistency and 5) publication bias (Murad et al, 2017). Implementation Fidelity (IF) using Carroll et al (2007) multifaceted framework was conducted to allow assessment of methodological practices of the intervention. This ensured the research study reliably and validly tested the implemented clinical intervention (Bellg et al, 2004). The assessment conducted under elements of; 1) adherence, encompassing content, coverage, frequency and duration; 2) moderation, covering intervention complexity, facilitation strategies, quality delivery, participant responsiveness; and 3) indication of essential components. The assessment was done by NN checked by RM and GA.

Results

Search results

A total of 437 studies were retrieved from the 5 databases and 1 from Clinical Trials.gov. After title and abstract screening 7.8% (n=34) studies met the inclusion criteria. Of these, 76% (n=26) were excluded at full screening stage; 9 conference abstracts with no full published paper, 3 were published in foreign languages, 11 due

to methodological/outcome exclusion, 2 had no available full publication that was confirmed by a librarian and 1 protocol with a study still in progress. Therefore, 24% (n=8) of studies were included in the review (figure 1). Hand search in the reference list of the included articles resulted in retrieval of any new relevant articles. Similarly, there were no new relevant publication from the final search conducted in August 2018.

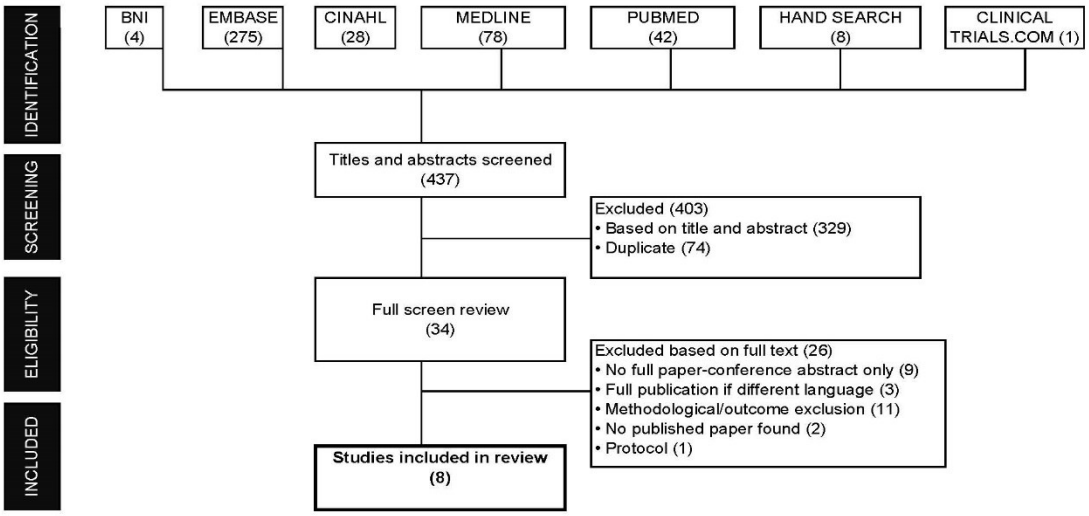

Figure 1: PRISMA flow diagram of summary of search results (PRISMA, 2009)

Characteristics of included studies

Country of origin and year of publication: The majority of the studies (n=6) were from Europe; Spain (Lillo et al, 2012; Romero et al, 2012; Romero et al, 2017); Turkey (Yazar et al, 2016); Austria (Cadamuro et al, 2016) and Sweden (Bolenius et al, 2013). The remaining 2 were from Australia, (Cockrill, 2012) and Singapore (Ong et al, 2008; 2009). All the studies were conducted between 2008 and 2017.

Study setting and participants' demographics: Studies were conducted in a hospital setting (Cadamuro et al 2016), an emergency department (Cockill, 2012; Ong et al, 2009), a laboratory (Lillo et al, 2012) and emergency clinics and polyclinics and services (Yazar et al, 2016). The remaining three studies were conducted in primary health care centres (Bolenius et al, 2013; Romero et al, 2012; Romero et al, 2017).

The majority of the studies (n=6) samples staff involved in the conduct of venepuncture. These included registered and enrolled nurses and laboratory technicians (Bolenius et al, 2012); junior doctors and nurses (Cadamuro et al, 2016); doctors and medical students (Ong et al, 2009); nurses and phlebotomists (Romero et al, 2012); nurses, physicians, auxiliary and administrative staff (Romero et al, 2017); and phlebotomists, nurses, paramedics, and other unspecified relevant personnel (Yazar et al, 2016). Cockill (2011) involved emergency department (ED) staff, with no specific details. Lillo et al (2012) sampled nurses.

Study design: None of the included studies were randomised controlled trials (RCTs). Designs used included prospective, before and after designs (Bolenius et al, 2013; Lillo et al, 2012; Romero et al, 2012; Yazar et al, 2017), observational cohort (Cadamuro et al 2016), quasi-experimental (Romero et al, 2012) and Quasi experimental using time series approach (Cockill, 2012); and phased prospective, before and after (Ong et al, 2008 and 2009) study.

Study quality

An evaluation of quality McMaster critical appraisal tool revealed risk of sample bias due to inclusion of staff of different disciplines with limit detail given on the numbers enrolled versus those participated in the intervention (ie Yazar et al, 2016). Variability

in the details on the intervention delivered (Yazar et al, 2016; Lillo et al, 2012), time delivered in some intervention (Cadamuro et al, 2016) and qualification of facilitators (Romero et al, 2012). Methodological problems in the research designs could have led to bias. While inconsistencies in some studies making it impossible to conclude if the outcome was due to the intervention or not impacting on the implementation fidelity (table 2).

Table 2: Assessment of methodological quality

Elements	Studies								
	1	**2**	**3**	**4**	**5a**	**5b**	**6**	**7**	**8**
Study Purpose									
Was the purpose stated clearly?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Literature									
Was relevant background literature reviewed?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Design									
Was the study design relevant to the research question?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sample									
Was the sample described in detail?	Y	Y	N	N	Y	Y	Y	Y	N
Was sample size justified?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Outcome									
Were the outcome measures reliable?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Intervention									
Was the intervention described in detail?	Y	Y	Y	N	Y	Y	Y	Y	Y
Was the content of intervention given?	Y	Y	Y	Y	Y	Y	N	Y	N
Was contamination avoided?	N	Y	Y	N	N	Y	N/A	N/A	UC
Was cointervention avoided?	N/A	UC	N/A	N/A	N/A	N/A	N/A	N/A	N
Were results reported in terms of statistical significance	Y	Y	Y	N	Y	Y	Y	Y	Y
Were the analysis method(s) appropriate?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Was clinical importance reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were drop-outs were reported?	Y	UC	UC	UC	UC	UC	N/A	N/A	N/A
Conclusion and Implications									
Did the conclusion appropriately addressed study method and results	Y	Y	Y	Y	Y	Y	Y	Y	Y

Key: Y = Yes; **N** = No; **UN** = Unclear; **N/A** = Not Applicable

Studies: 1. Bolenius et al (2012); **2**. Cadamuro et al (2016); **3**. Cockill (2011); **4**. Lillo et al (2012); **5a**. Ong et al (2009); **5b.** Ong et al (2008); **6.** Romero et al (2012); **7.**Romero et al (2017); **8.** Yazar et al (2016)

GRADE assessment: None of the included studies were RCTs. Five of the studies (Bolenius et al, 2012; Cadamuro et al, 2016; Cockill, 2012; Ong et al, 2009; Yazar et al, 2016) investigated the impact of training on a single primary outcome (haemolysis rate). Three involved multiple pre-analytical errors (ie rate of clotted and insufficient samples and impact on use of quality labels) besides haemolysis rate (Lillo et al, 2012). Similarly, Romero et al (2012) and Romero et al, (2017) had multiple outcomes (ie missed sample, incorrect volume) and haemolysis rate. One study (Ong et al, 2009) was clear on the study limitation (table 3).

Implementation Fidelity assessment: There was heterogeneity in the intervention delivered with some of the studies using available international guideline (CLSI, 2007 and WHO, 2010) on the conduct of venepuncture. Cadamuro et al (2016) performed individual training based on standard operating procedure (SOP) and practice on mannequins according to Greiner BioOne, Austria Company. This was supervised by 4 train-the-trainers. Equally, training can successfully and meaningfully be delivered if only the essential components of the model are implemented to meet local needs; identified through, either sensitive or combined components analysis (Carroll et al, 2007).

Both Corkill (2012) and Ong et al (2009) had high reduction after implementing targeted interventions in local EDs based on essential component of 'content'. In contrast, Bolenius et al (2013) intervention included essential component on 'participants; responsiveness' by examining participant on prevention of PABSH, this had negative results.

Table 1: Rating certainty of evidence using GRADE Framework*

GRADE Domain	Judgement	Concerns about certainty domains
Methodological limitations about studies	None of the included studies were RTCs; 5 out of 8 studies (1, 4, 5, 6, 8) having used single arm prospective before and after design. Two studies (3 and 7) used quasi experimental design and one (2) used cohort. Only one study (5) exclusively discussed the limitations, highlighted recommendations for further and gave reasons these were not implemented the current study. Three investigated impact of training on multiple outcomes in addition to haemolysis rate, outcomes relating to sample quality labels; availability of sample for analysis, whether the sample was clotted, insufficient samples (4); missed sample, incorrect volume, and other mistakes (6, 7) were studied. Overall, we have judged the studies to have serious methodological limitations.	Serious
Indirectness	There was heterogeneity in the participants to the eight studies who ranged from administrative staff (7) to laboratory technicians (1), medical students (5), auxiliary staff (7) and professional staff of different disciplines and grades (1-8). All interventions included education of varying description of the content with one (8) not clearly elaborating the content or source. There were differences in the detection of haemolysed samples, one (3) had used visual detection, three (1, 2, 7). These latter three studies had different set threshold for detecting haemolysed samples, 0.15g/L (1), 0.25 (7) and 0.5g/L, 1g/L (2). The other four studies did not give the set threshold used (4, 5, 6, 8). Therefore we judged the evidence to have very serious indirectness.	Very serious
Imprecision	Six studies from our review were from Europe (1, 2, 4,), 1 from Asia (5) and one from Australia (3) the exclusion of publication in other languages meant representation of that population. There were no studies from Africa and South America. Other staff disciplines were under represented ie laboratory technicians. We judge the evidence to have borderline imprecision.	Borderline
Inconsistency	Time spent delivering the intervention ranged from 25 hours (5) to 4 months (3); with intervention varying from available evidence (3, 5), some based on available guidelines (1, 2, 3). There were varying outcomes; some studies (3, 4, 5, 8) reported positive reduction in haemolysis rate after intervention while others (1, 6) had negative impact and the remaining two studies (2 and 7) had equivocal results. We judged the evidence to have serious inconsistency.	Serious
Publication bias	Publication bias was not suspected with both studies with negative and positive outcome published; and there was extensive search for studies.	Not suspected
Included studies	**1**= Bolenius et al (2013); **2** =Cadamuro et al (2016); **3** = Cockill (2011); **4** = Lillo et al (2012; **5** = Ong et al (2008; 2009); **6** = Romero et al (2012); **7** = Romero et al (2017); **8** = Yazar et al (2016). *GRADE Framework adopted from Murad et al, 2017	

Lack of sensitive and component analysis (Carroll et al, 2007) could have contributed to the failure. However, effect of management's restrictions on the length of the intervention delivery could not be excluded. Conversely, Cadamuro et al (2016) used the 'coverage' component. As in Bolenius et al (2013) above, there was lack of sensitive and component analysis.

Education and training and delivery time: There were variabilities in the quality of intervention and its pellucidity. Cadamuro et al (2016) and Lillo et al (2012) were the only two studies which included phlebotomy in the training; with general content or none given respectively. While there was no clarity in the content used in the intervention (Yazar et al, 2016), comparing the effects of intensive training versus routine training; others used more than one intervention (presentation and examination) Bolenius et al (2013); and presentation and practical (Cadamuro et al, 2016). Cockill; (2012); and Ong et al (2009) used essential components of 'content' based on available evidence. Lillo et al (2012) used expert support in the conduct of venepuncture including mentoring and an information leaflet for the first month of the new nurses arriving in the department. There were differences in the design and implementation of the intervention; with presentation being the most used mode. Cadamuro et al (2016), Yazar et al (2016), Bolenius et al (2013) Lillo et al (2012) added other delivery (ie practical, examination and mentoring) to the presentation.

The shortest delivery time of intervention was 0.25 hour (h), in which the common causes of hemolysis, based on available local evidence, were presented and discussed in brief (Ong et al, 2009). Romero et al, (2012) and Romero et al, (2017) spent an hour on update presentation supported by leaflets that were developed

based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (Romero et al, (2017). The longest intervention was 4 months conducted through education toilet posters designed based on available evidence (Cockill (2012). Bolenius et al. (2013) used a 2-h lecture followed by 5 written examination questions, an acceptable performance on which led to certificate of competence. Cadamuro et al (2016) delivered a 2 hour education session and individual training based on WHO and CLSI recommendations on phlebotomy training and practice on mannequins (appendix 1).

Haemolysis Rate threshold: Four (50%) of the reviewed studies reported the haemolysis index (HI) threshold; however, the identification, set ranges and units used were variable. Bolenius et al (2013) gave the measurement of HI (>15 to >100) with corresponding fHb (150 to 1000mg/L) depending on analytes of measurement. Similarly, Cadamuro et al (2016) reported HI (>50 to 100) and corresponding fHb (>500 to 1000mg/L). Different analytical platforms (ie Beckman Unicel DxC600i analyser, Beckman-Coulter DxC 800, Beckman Coulter Inc., Fullerton, California; Dimension Vista 1500, Siemens, Malvern PA, USA; Cobas 8000, Roche Diagnostics, Basel, Switzerland); were mentioned in some of the papers.

Both the visual dictation absorbance method (score 1 to 6) and a set threshold of fHb (250mg/L) were used in Romero et al (2017); however there was no meaning given to the scores. In contrast, gave Cockill (2012) used similar system (0 to 10+) with benchmark set at HI >3; similarly, no corresponding scale for fHb was given. Goyal and Scmottzer (2015), gives corresponding value of HI greater than 3 as 26-50mg/dL fHb in a Siemens Dimension Vista Chemistry Analyzer. All units in this review were

converted to g/L to allow comparison of threshold (table 4). None of the studies had a pre-set threshold for clinical/practical importance for which the intervention was aimed to achieve.

Low pre-interventional HR (0.2% to 10%), was observed in the studies conducted in Europe, n=5, (Romero et al, 2017; Cadamuro et al, 2016; Yazar et al, 2016; Bolenius et al, 2013; Romero et al, 2012). In comparison to Ong et al (2009), an Asian study (19.8%). However, Cockill (2012), an Australian study, reported no baseline rate. Lack of standardisation in setting of fHb level to detect haemolysis is reported in McCaughey et al (2016).

Findings: Seven studies (88%) reported the HR pre and post intervention (Bolenius et al, 2013; Cadamuro et al, 2016; Romero et al, 2017; Lillo et al, 2012; Romero et al, 20012; Ong et al 2009; Yazar et al, 2016). The highest reduction (19.7%) was reported in Cockill (2012) after toilet based education posters from available evidence. Similarly, Ong et al, (2009) had the next reported highest reduction rate (from 19.8% to 4.9%, p=0.001) after presentation and discussion based on local findings. In comparison, Yazar et al (2016) reported lowest reduction rates (0.22% to 0.07%; 0.27% to 0.18%) after compering effect of intensive to routine training respectively. Similarly, Lillo et al (2012) reported lowest reduction (0.2 - 0.013%) after mentoring and supervision.

Table 4: Pre and post haemolysis rates and threshold

Author(s)	Year	Country	Haemolysed samples						Threshold
			Pre-intervention			Post-intervention			
			Percentage (%)	Number (n)	Total	Percentage (%)	Number (n)	Total	
Bolenius et al	2013	Sweden	10.5	698	6652	11.8	722	6121	0.15g/L
Cadamuro et al	2016	Austria	1.8	387	21512	1.6	358	22363	>0.5g/L
			0.6	129	21512	0.6	134	22363	>1g/L
Cockill	2011	Australia	Presented statistical results only						>3[∞]
Lillo et al	2012	Spain	0.2*	90	44896	0.013	30	15444	Not given
Ong et al	2009	Singapore	19.8	45	227	4.9	10	204	Not given
Romero et al	2012	Spain	1.97	1408	71472	3.9	2835	72692	Not given
Romero et al	2017	Spain	2.42	3592	111806	1.61	3682	132755	
			0.35	341	69942	0.43	955	132235	0.25g/L
Yazar et al	2016	Turkey	0.27	346	129297	0.18	239	37560	
			0.22	81	37549	0.07	25	129301	Not given

[∞] No equivalence was given; *figures given per 10000, converted per 100

There were unclear results after an hour of clinical update sessions on pre-analytical errors to multidisciplinary teams (Romero et al, 2017). A reduction in HR (3.21% to 2.77%, p=<0.05) in samples sent to laboratory 1 and a rise (0.48% to 0.72%, p=<0.05) in samples sent to laboratory 2 (Romero et al, 2017). Cadamuro et al, (2016) reported similar results with a reduction (1.8% to 1.6%, p=0.021) in samples with fHb 0.5g/L, and no change (0.6% to 0.6%, p=0.221) in samples with fHb >1g/L after a 2 hour phlebotomy and individual training.

Discussion

The evidence synthesised in our review leads us to conclude that the effectiveness of venepuncture training on the reduction of PABSH rate is unclear at present. A lack of RCTs undermines the quality of the evidence; and the variability between studies especially in haemolysis threshold definitions precluded a robust quantification of any training intervention effect on PABSH.

The absence of RCTs rendered the evaluation of the effectiveness of intervention in the reduction of PABSH rate unreliable. Most studies were single group pre-post designs. Therefore, threats to validity such as regression to the mean cannot be ruled out. While the implementation of RCTs might be difficult in some contexts it is conceivable that a cluster RCT, with randomisation at the level of clinic or hospital, may be possible to investigate the effects of specialist staff training on haemolysis rates.

There was heterogeneity across the studies in terms of the content of the implemented intervention, the population of interest, the mode of delivery and time spent delivering and monitoring the content, which could have undermined the implementation fidelity.

According to Carroll et al (2007) such variability in the intervention would make the understanding and quantification of such intervention effects difficult as well as unlikely to be replicated.

We also identified differences in haemolysis threshold setting as another obstacle to a quantitative synthesis. While the automated identification of haemolysis (from the results of the analyser) may discourage subjectivity in detection of haemolysed samples, there seems to be a lack of standardisation in threshold setting between different analysers. These inconsistencies have also been reported by Lippi et al (2009) and Shin et al, (2014). Between-analyser variability has been especially reported for the ADVIA 2400 and 1800 Dimensions (Lippi et al, 2009). It was also reported that the qualitative or quantitative HI on most instruments gives the users the flexibility to adjust the levels at which the interference generates a red flag; enabling them to customize the operating mode to reflect individual operating requirements for reporting interference (Lippi et al 2009). This inevitably may lead to variability in HI and resulting resampling rates between clinics and hospitals, especially in different countries. Nevertheless, Dolci and Panteghini (2014) argued that variability in the parameters set by the manufacturers in estimation of HI on different analysing platforms were sometimes clinically insignificant; such that the maximum haemoglobin concentration set corresponds to the highest HI value, ranging from 5 to 20 g/l; while haemolysis inducing release of haemoglobin up to 10 g/l is clinically common (Dolci and Panteghini, 2014).

There are national and international initiatives being implemented, such as external quality assessment schemes, e.g., The National External Quality Assessment

Services, to ensure the thresholds are at acceptable levels. Such initiatives provide common reference lists in comparison to the recommended decision thresholds, based on clinical outcome studies; constituting the highest level of quality (Tate et al, 2014). The Joint Committee for Traceability in Laboratory Medicine (JCTLM) comprises experts representing the clinical laboratory profession, government agencies, and manufacturers; it is through this group that values have been assigned to kit calibrators with consistency checked using appropriate higher order reference materials (Armbruster and Miller, 2007). Nevertheless, the JCTLM does not appear to have a formal governmental budget and membership is voluntary (Armbruster and Miller, 2007). This may slow down efforts to standardise haemolysis detection thresholds.

Limitations and Recommendation

The research designs used in included studies in our review detrimentally affected the quality of evidence. Our review highlights the shortfall currently facing the venepuncture services; of greatest priority has been lack of standardisation in threshold setting that may have a clinical significance on the patient safety, quality of care and cost. Although initiatives are currently being implemented, there is a need for clinical outcome studies for threshold setting. Decision thresholds based on clinical outcome studies constitute the highest level of quality, with the clinical expectation that all methods employed in the clinical setting are harmonized; in comparison to reference limits based on an assay's kit insert data constituting the lowest quality (Tate et al, 2014). Therefore, multi stakeholder involvement is crucial in the subject. These would include multi-centre, multi-stakeholder RCTs that will generate high quality evidence applicable to all the highlighted areas of great interest.

Conclusions

Our review has confirmed lack of robust evidence in the research area. The variability in the in the participants across the studies, differences in the interventions, the mode of delivery and time spent, impacted on the overall quality of the evidence, on implementation fidelity. We therefore conclude that the effects of specialist training on clinic haemolysis rates are unclear because of a lack of RCTs and variable detection thresholds for haemolysis between studies. Our review has also highlighted the complexity facing venepuncture services in general and the training aspects in particular, in facilitating reduction in PABSH rate. While efforts are being implemented to address harmonisation of threshold setting internationally, limitations such as none compulsory participation to the JCTLM Consortium and lack of budget may impede on the progress that could be made.

Relevance to Clinical Practice

More randomised controlled trials are needed to confirm the effectiveness of venepuncture training programmes for reducing haemolysis rates in clinic. Ideally, haemolysis thresholds would also be standardised across countries to enable more robust quantitative syntheses of the various study findings.

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Appendix 1: Characteristics of included studies

	Methodology			Intervention			Outcome
Study	Setting	Design	Sample and size	Type	Mode	Duration (hours)	Key findings
Bolenius et al (2012)	PHC	Prospective before/after	120 RGNs, 31 ENs, 1 laboratory technician	education on VBSC according to national and local guidelines	large scale lecture and 5 written examination questions leading to competence certificate	2	Percentage of sample HI $\geq$ 15 (approximately 150mg/L) increased post intervention compared to pre-intervention. Rural PHC had improved pre and post haemolysis percentage compared to the Urban PHC.
Cadamuro et al (2016)	IP	Observational (Cohort)	70 Junior doctors and 874 nurses	Training and increased number of phlebotomists	Educational sessions and individual training based on WHO and CLSI recommendations phlebotomy training on demo arms under supervision of experienced trainer.	2	Number of haemolysed samples (HI >0.5g/L fHb) was significantly higher when clinicians were in charge of phlebotomy. There was no change in the number of haemolysed samples HI>1g/L fHb
Cockill (2011)	ED	Quasi experimental	ED staff details and numbers not given	Educational posters	Toilet Education Posters on evidence on minimising haemolysis	2880	There was a reduction in samples with HI - post intervention based on prior identified causes of haemolysis
Lillo et al (2012)	OP	Prospective before/after	Nurses, details and number not given	Leaflet and mentoring	Informative leaflet on phlebotomy and expert support	720	There was reduction in all pre-analytical indicators with the highest reduction in the haemolysis rate

Ong et al (2009)	ED	Phased Prospective before/after	Doctors and medical students details and numbers not given	Education program	Presentation and discussion	0.25	An educational program based on prior research results led to significant reduction in haemolysis rate in ED
Romero et al (2012)	PCC	Prospective before/after	240 PC nurse phlebotomists	Educational sessions	Series of clinical updates	1	There was a rise in haemolysed samples after the intervention
Romero et al (2017)	PCC	Quasi experimental	941 nurses, 906 physicians, 647 auxiliary/administrative staff	Educational sessions; leaflets	Series of multidisciplinary clinical update sessions on pre-analytical procedures	1	HI set at 250mg/L.There was variability in the reduction of haemolysis rate between the two laboratories after the intervention. The rate reduced in the rate in Laboratory 1 (from 3.21 to 2.77%) and an increase in Laboratory 2 (from 0.48 to 0.72%).
Yazar et al (2016)	OP/ IP	Prospective before/after	Phlebotomists, nurses, paramedics and other relevant personnel, numbers not specified	Intensive (IT) versus Routine training (RT)	IT included training twice a day and unannounced observations four times a day RT included training delivered in a single day followed by once a month audit	Not specified	Haemolysis rate had reduced in both groups post training. The reduction was greater in the group that had gone through IT compared to RT

KEY: IP=In patient; OP=Outpatient; PHC=Primary Health Centre; PCC= Primary Care Centre; ED=Emergency Department; RGN= Registered General Nurse; EN =Enrolled Nurse.