Research paper

‘True Blood’ The Critical Care Story: An audit of blood sampling practice across three adult, paediatric and neonatal intensive care settings

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Article history:
Received 17 March 2015
Received in revised form 30 April 2015
Accepted 10 June 2015

Keywords:
Blood conservation strategies
Blood specimen collection
Critical care
Evidence based nursing
Nursing research

ABSTRACT

Background: Anaemia is common in critically ill patients, and has a significant negative impact on patients’ recovery. Blood conservation strategies have been developed to reduce the incidence of iatrogenic anaemia caused by sampling for diagnostic testing.

Objectives: Describe practice and local guidelines in adult, paediatric and neonatal Australian intensive care units (ICUs) regarding blood sampling and conservation strategies.

Methods: Cross-sectional descriptive study, conducted July 2013 over one week in single adult, paediatric and neonatal ICUs in Brisbane. Data were collected on diagnostic blood samples obtained during the study period, including demographic and acuity data of patients. Institutional blood conservation practice and guidelines were compared against seven evidence-based recommendations.

Results: A total of 940 blood sampling episodes from 96 patients were examined across three sites. Arterial blood gas was the predominant reason for blood sampling in each unit, accounting for 82% of adult, 80% of paediatric and 47% of neonatal samples taken (p < 0.001). Adult patients had significantly more median [IQR] samples per day in comparison to paediatrics and neonates (adults 5.0 [2.4]; paediatrics 2.3 [2.9]; neonatal 0.7 [2.7]), which significantly increased median [IQR] blood sampling costs per day (adults AUD$101.11 [54.71]; paediatrics AUD$41.55 [56.74]; neonatal AUD$8.13 [14.95]; p < 0.001). The total volume of samples per day (median [IQR]) was also highest in adults (adults 22.3 mL [16.8]; paediatrics 5.0 mL [1.0]; neonates 0.16 mL [0.4]). There was little information about blood conservation strategies in the local clinical practice guidelines, with the adult and neonatal sites including none of the seven recommendations.

Conclusions: There was significant variation in blood sampling practice and conservation strategies between critical care settings. This has implications not only for anaemia but also infection control and healthcare costs.

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1. Introduction

1.1. Background

Anaemia is common in critically ill patients admitted to the ICU, with almost 95% of patients having an abnormally low haemoglobin level by ICU day three. The damaging effects of anaemia include increased risk of cardiac morbidity and mortality, as well as a generalised decrease in oxygen carrying capacity to the organs and tissues. Critically ill patients are at particular risk for adverse consequences from anaemia given the cardiovascular, respiratory and metabolic compromise frequently encountered during critical illness.

The aetiology of anaemia during critical illness is multifactorial. Its severity is influenced by frequent phlebotomy, sepsis, gastrointestinal bleeding, coagulation disorders, blood loss from vascular procedures, renal failure, nutritional deficiencies, bone marrow suppression and impaired erythropoietin response. For at least 40 years medical literature has highlighted the importance of an iatrogenic contribution to the anaemia seen in hospitalised patients due to blood sampling, and its potential negative impact upon recovery.

Blood samples from critically ill patients are routinely collected via arterial and central venous access devices, by peripheral venepuncture or heal/finger prick. Blood draws from intravascular devices increase blood loss due to the need to first withdraw a clear or ‘discard’ volume from the device, to ensure the resultant sample is whole blood and not partially medication or infusion fluid. Monitoring of blood flow, acid-base status, oxygen transport, coagulation, visceral organ function and the development of healthcare associated infection are a few of many reasons for diagnostic blood testing. Previous reports of blood removed from critically ill adult patients for testing average between 41.5 mL and 377 mL per day. The described daily average blood sampling volumes varied depending upon the population studied, the length of stay evaluated and the methodology of the study; with the highest volumes commonly occurring in the immediate post-operative period.

Just over a decade ago, seminal work by Vincent et al. and Corwin et al. described the challenges associated with blood conservation practices throughout ICUs and the resulting over-prescription of packed red blood cell (PRBC) transfusions. Current evidence suggests that PRBC transfusions are associated with infectious and inflammatory complications, significant financial costs, worse clinical outcomes and transfusion errors. A recent Australian retrospective cohort study described the annual total hospital-associated cost of PRBC transfusions as AUS$7 million; with the inpatient costs of those who received a blood transfusion 1.83 times higher than those not transfused, after adjusting for confounders. The use of PRBC remains a significant financial burden on the Australian healthcare system. Because of these burdens and risks, the National Health and Medical Research Council have championed the development of clinical protocols across health-care facilities to minimise and direct the correct use of blood products and other supportive therapies.

While phlebotomy and blood testing to inform clinical decision making is vital, strategies have been developed to minimise unnecessary iatrogenic blood loss. Clinical practice strategies and technologies available in Australia include closed-system sampling enabling safe return of arterial and central line clearing volumes to the patient, small-volume phlebotomy tubes, frequent clinical evaluation of routine or repetitive testing, use of noninvasive methods where possible (e.g. end tidal carbon dioxide [ETCO₂], oxygen saturations [SpO₂]), bundled scheduling of blood tests to minimise loss of ‘clearing’ volume, routine charting of cumulative daily phlebotomy blood loss, and point of care bedside microanalysis. Randomised controlled studies and clinical controlled trials have been undertaken surrounding the efficacy of individual conservation strategies to prevent and/or treat the associated anaemia, including the use of small-volume phlebotomy tubes, closed-system sampling enabling safe discard return and a combined approach. Other strategies commonly advocated in clinical settings, such as point of care bedside microanalysis, have less rigorous observational studies to support their use. The evidence to support and encourage the use of these blood conservation strategies in critical care settings has not been summarised in international clinical practice guidelines (CPG), such as the CPG developed for the prevention of cather-related bloodstream infection. Instead, clinicians are guided by the provision of local CPGs, developed within the hospital or ICU based on varied quality of evidence, often combining peer-reviewed research, local tradition and expert opinion.

Although phlebotomy amounts can be dramatically reduced by the use of blood conservation strategies, research suggests they are not widely practiced in all adult, paediatric and neonatal ICUs. Landmark studies describing the importance of blood conservation strategies to prevent iatrogenic anaemia for critically ill patients were published almost a decade ago. Our study aim was to investigate the blood conservation practice across ICUs in Australia and their direct financial consequences.

1.2. Objectives

There were three study objectives:

1. To describe current blood sampling practices in adult, paediatric and neonatal ICUs;
2. To provide an estimate of direct pathology costs associated with blood sampling practices in adult, paediatric and neonatal ICUs; and
3. To compare local CPG and current practice regarding blood conservation strategies in adult, paediatric and neonatal ICUs, with international evidence-based recommendations.

2. Methods

2.1. Study design

A cross-sectional, descriptive study was completed over one week in July 2013.

2.2. Participants and setting

Blood sampling practice was audited within three Queensland ICUs: the adult ICU at the Royal Brisbane and Women’s Hospital (RBWH), Brisbane, Australia; the paediatric ICU at the Royal Children’s Hospital (RCH), Brisbane, Australia; and the neonatal ICU at the RBWH, Australia. Each of the ICUs are tertiary-referral centres for the area. Data were collected on all inpatients in the three ICUs on each of seven days over one week. There were no other inclusion or exclusion criteria. University and hospital ethics approval was gained for this study (HREC/13/QRCH/32 and GU: NRS/21/13/HREC).

2.3. Blood sampling audit

2.3.1. Data collection and measurement

In order to describe blood sampling practice in the critical care settings, the main outcomes collected were the amount, frequency and type of blood sampling from all patients during the audit period. To quantify these outcomes, an audit was developed and
each nurse for every shift documented the amount and reason for each blood sampling episode. Demographic and clinical variables for the patients were recorded to examine for association with the main outcomes. The variables included age, severity of illness, ICU length of stay, primary diagnosis, ventilation, renal replacement therapy and ICU outcome. The data collected were based on the outcomes and variables reported in previous studies. Severity of critical illness was estimated using paediatric logistic organ dysfunction 2 (PELOD2) score for neonates and paediatrics and acute physiology and chronic health evaluation II (APACHE II) for adults. Both are cumulative scores based on clinical and biological measurements, with higher scores indicating higher levels of critical illness and risk of mortality.

All data were collected on locally adapted data collection tools but using the same variables. Locally based study coordinators oversaw the data collection by bedside nurses for the audit period at each study site. Clinicians were educated about the data collection tool and research project by the study coordinator using one-on-one and group session education. The study coordinator was available at all times during the audit period, to support clinician compliance. Prior to the audit period, the tool and data collection process were piloted for feasibility and utility at each site for a single day and amendments made accordingly. Costing of blood sampling was based on the pricing in the Medicare Benefit Schedule for arterial blood gas (ABG), capillary blood gas, full blood count, urinary electrolytes, liver function tests, coagulation studies, cross match and c-reactive protein.

2.4. Blood conservation CPG

Local hospital guidelines and ICU CPGs regarding blood sampling and conservation were appraised for specific recommendations regarding rationale, process, frequency and volume of sampling. Local hospital and ICU CPG were provided via the ICU manager. These included local policies, procedural guidelines, protocols, manuals, nursing standards or work instructions related to blood sampling and conservation. Each were reviewed and assessed for content and incorporation of seven evidence-based blood conservation strategies which are recommended in peer-reviewed literature. These strategies were: frequent evaluation by clinicians of routine blood sampling orders, closed-system sampling, small-volume phlebotomy tubes, non-invasive monitoring, bundled scheduling of blood sampling, charting of cumulative daily phlebotomy loss and point of care testing.

2.5. Statistical methods

Data were entered and analysed using PASW Statistics Version 21.0 (SPSS Inc., Chicago, IL, USA). Basic frequencies were calculated for all variables and any extreme or obviously incorrect data were re-checked for accuracy. Blood sampling practices were described using descriptive statistics. Continuous variables were summarised using mean with standard deviation (SD) or median with interquartile range (IQR) depending on normality of distribution. Categorical data was summarised by frequencies and percentages. Pathology cost associated with blood sample across ICUs were summarised as median costs with IQR per patient per day and per patient per admission. These were described for ABGs and total pathology costs (a composite of all pathology tests including ABG, capillary blood gas, full blood count, urinary electrolytes, liver function tests, coagulation studies, cross match and c-reactive protein). Potential associations between intensive care settings and frequency, volume and pathology costs of blood sampling were assessed by chi square, Mann–Whitney, Kruskal–Wallis, t-tests or analysis of variance (ANOVA). Statistical results of \( p < 0.05 \) were considered significant. All missing data are explained in the tables and within the results section.

3. Results

3.1. Sample

A total of 940 samples from 96 patients were examined; 100% of patients admitted during the study period. Individually, 655 samples were examined from 50 patients in the adult ICU, 145 samples from 16 patients in the paediatric ICU and 140 samples from 30 patients in the neonatal ICU.

3.2. Descriptive data

Table 1 provides the demographic characteristics of the participants. The majority of the participants in each ICU were mechanically ventilated and admitted to the ICU for at least 50 h during the study period. As expected from a heterogeneous study population, the participants had a variety of age, primary diagnosis, severity of critical illness and admission sources. The PELOD2 scores for the paediatric ICU and neonatal ICU populations indicated low to moderate levels of organ dysfunction. The APACHE II score for the adult ICU population indicated a moderate level of dependency and risk of mortality.

3.3. Blood sampling practice

The individual characteristics of the blood sampling practices between the ICUs are outlined in Table 2. ABG were the major reason for blood sampling in each ICU accounting for 82% for samples in adults, 80% in paediatrics and 47% in neonates \( (p < 0.001) \). There was 492/945 (52.1%) missing data on the reason for blood sampling. Of data received, the main reason for blood sampling across ICU settings was routine or medical doctor requested (adults 93%; paediatrics 99%; neonates 90%, \( p < 0.024) \). ABGs were more often nurse initiated in the adult (49%) and paediatric ICUS (53%), as opposed to the neonatal ICU which were most commonly due to medical

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**Table 1**

<table>
<thead>
<tr>
<th>Clinical characteristics of the ICU patients across study sites (n = 96).</th>
<th>Neonatal ICU (n = 30)</th>
<th>Paediatric ICU (n = 16)</th>
<th>Adult ICU (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y):</td>
<td>8.4 (3.9)</td>
<td>33.2 (19.1)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wks):</td>
<td>30.5 (5.4)</td>
<td>23.2 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Weight (g):</td>
<td>1518 (1264)</td>
<td>2020 (275)</td>
<td></td>
</tr>
<tr>
<td>APACHE II:</td>
<td>6.9 (0.7)</td>
<td>3.5 (3.3)</td>
<td></td>
</tr>
<tr>
<td>PELOD2:</td>
<td>129.7 (61.9)</td>
<td>74.1 (51.5)</td>
<td>64.1 (60.9)</td>
</tr>
<tr>
<td>Study ICU LOS (h):</td>
<td>18 (60%)</td>
<td>8 (50%)</td>
<td>30 (60%)</td>
</tr>
<tr>
<td>Gender: Male:</td>
<td>24 (80%)</td>
<td>3 (19%)</td>
<td></td>
</tr>
<tr>
<td>Primary diagnosis: Respiratory:</td>
<td>31 (19%)</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td>Post-operative:</td>
<td>0 (0%)</td>
<td>22 (44%)</td>
<td></td>
</tr>
<tr>
<td>Trauma:</td>
<td>3 (19%)</td>
<td>16 (7%)</td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td>6 (30%)</td>
<td>15 (30%)</td>
<td></td>
</tr>
<tr>
<td>Ventilated during ICU admission:</td>
<td>28 (93%)</td>
<td>8 (50%)</td>
<td>30 (60%)</td>
</tr>
<tr>
<td>Continuous renal replacement therapy during ICU admission:</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

* Mean (standard deviation); APACHE, acute physiology and chronic health evaluation; LOS, length of stay; PELOD, paediatric logistic organ dysfunction.
3.4. Pathology costs associated with blood sampling

Median pathology costs (2014 AUD, Queensland Pathology) per ICU type for processing ABGs were between AU$5.25 (neonatal ICU) and AU$55.51 (adult ICU) per patient per day (Table 3). Total pathology costs (all blood tests) were between AU$8.31 (neonatal ICU) and AU$101.11 (adult ICU) per patient per day (Kruskal–Wallis p < 0.001). These costs do not include nursing staff time to take blood tests nor for blood sampling equipment used in the ICU.

3.5. Blood conservation strategies in local CPG and in practice

Each of the ICUs had a local CPG regarding blood sampling, but content varied in the inclusion of evidence-based blood conservation strategies (see Table 4). The neonatal and adult ICU had specific procedural guidelines for the use of arterial (adult and neonatal) and umbilical lines (neonatal only) and their sampling, but not for sampling from other sources (e.g. CVADs or heel pricks). The paediatric ICU used local “nursing standards” in combination with the medical registrar staff manual. Only the paediatric ICU contained any of the evidence-based recommendations in their local CPG, while all ICUs used non-invasive monitoring and point of care testing within their practice.

4. Discussion

This study achieved its aim to describe blood sampling practice across critical care settings, provide costings of tests and audit local guidelines for sampling processes and evidence-based recommendations. Demographics of each population demonstrated a representative and comparable level of critical illness. Proxy measures of critical illness (mechanical ventilation, gestational age and weight, length of stay in ICU and outcome) indicated a potentially critically ill population in each of the ICU sites, including the neonatal ICU.

Our study found a lower median volume of blood samples per adult patient per day (38 mL) compared to previous studies in adult ICU populations (41.5–377 mL per day).1,2,6,12 This may be due to increased awareness surrounding the contribution and consequences associated with blood sampling and iatrogenic anaemia, as alerted in the landmark studies.2,3 Comparisons to previous paediatric or neonatal ICU studies could not be made, due to a lack of reported blood sampling values and research in this area.

Adult participants were sampled more frequently and using greater volumes, than the paediatric and neonatal participants. The reason for this variation was not adequately explained by comparison of severity of illness, as would be expected to inform treatment decision-making (e.g. electrolyte and blood product requirements). We were not able to directly compare illness severity between the study populations since there is no validated assessment or scoring tool to compare critical illness severity across the three age-related populations. However, proxy measures

### Table 2
Blood sampling across the included intensive care units (n = 940).

<table>
<thead>
<tr>
<th>Blood test:</th>
<th>Neonatal ICU (n = 140)</th>
<th>Paediatric ICU (n = 145)</th>
<th>Adult ICU (n = 655)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood gas</td>
<td>66 (47%)</td>
<td>116 (80%)</td>
<td>543 (82%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Full blood count</td>
<td>21 (15%)</td>
<td>38 (26%)</td>
<td>152 (23%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Capillary gas</td>
<td>35 (25%)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Urea, electrolytes and liver function test</td>
<td>9 (6%)</td>
<td>36 (25%)</td>
<td>157 (24%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>38 (27%)</td>
<td>55 (38%)</td>
<td>128 (20%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Table 3
Cost of blood sampling pathology processing across the included intensive care units in Australian (2014) dollars (n = 940).

<table>
<thead>
<tr>
<th>Blood test:</th>
<th>Neonatal ICU (n = 140)</th>
<th>Paediatric ICU (n = 145)</th>
<th>Adult ICU (n = 655)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ABCG cost per patient per admission</td>
<td>$24.48</td>
<td>$36.72</td>
<td>$73.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>(61.20)</td>
<td>(104.04)</td>
<td>(223.38)</td>
<td></td>
</tr>
<tr>
<td>Total Blood Sample costs per patient per admission</td>
<td>$28.75</td>
<td>$117.98</td>
<td>$128.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>(62.72)</td>
<td>(291.04)</td>
<td>(345.18)</td>
<td></td>
</tr>
<tr>
<td>ABG costs per patient per day</td>
<td>$5.25</td>
<td>$19.96</td>
<td>$51.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>(9.18)</td>
<td>(46.46)</td>
<td>(25.85)</td>
<td></td>
</tr>
<tr>
<td>Blood Sample costs per patient per day</td>
<td>$8.13</td>
<td>$41.55</td>
<td>$101.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>(14.95)</td>
<td>(56.74)</td>
<td>(54.71)</td>
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</table>
including mechanical ventilation, emergency admission type, and length of stay were more common in the neonatal population, in comparison to adults. Despite this, neonates had the lowest number of blood sampling episodes, and volumes drawn per day. If the patients’ clinical characteristics are not the cause of increased blood sampling frequency and volume, the underlying decision-making by clinicians is unclear. It is plausible that blood sampling practice remains a matter of tradition, clinician preference or fear, in comparison to a reflection of the best available evidence. Awareness of maintaining fluid balance and blood volume, is likely more prominent in the minds of neonatal ICU practitioners, with the risk of iatrogenic anaemia under-estimated in older children or adult patients.

The lower daily blood sampling volume in the paediatric and neonatal ICUs was partially explained by the routine use of small-volume phlebotomy tubes. Previous research by Smoller and colleagues compared the use of paediatric tubes with regular blood collection tubes in the adult health care setting, finding a 42% decrease in blood loss with the smaller tubes, without adverse outcomes or diagnostic inaccuracy. Within Queensland public health facilities, the actual sample required and the pathology costs associated with processing of small or standard-volume phlebotomy tubes is the same so it is unclear why these are not rolled out for all patient testing. The role for standard-volume phlebotomy tubes in the critically ill environment, where patients are at high risk for anaemia and its sequelae, is questionable.

While none of the included ICUs used ‘closed’ (in-line) arterial blood sampling systems, there was a variety in practice surrounding the discard of line clearance fluid. The neonatal population reported 100% of line clearance fluid was reinfused, while in adults 100% of line clearance fluid was discarded. There is little direct evidence regarding the safety of reinfusing line clearance fluid; and research that is available is conflicting with outcomes dependent upon the variety of additional technologies used (e.g. closed systems, needleless connectors). There are theoretical concerns regarding the reinfusing of line clearance fluid and the risk for hub contamination and thrombosis. There are some conditions for which the reinfusion of this fluid causes unacceptable risk of complication (e.g. hyper-coagulopathy, arterial spasm). Further evidence is needed in order to facilitate clinicians making an informed decision surrounding use of these technologies.

The study limitations include the small sample size, but it provides a snapshot of blood sampling and conservation information within these specific study centres. These results may not be generalisable to all ICUs, however the ICUs that participated in the study are likely somewhat indicative of non-cardiac tertiary Australian neonatal, paediatric and adult ICUs. Blood sampling practice was audited via self-report completed by the ICU nursing staff. This may have resulted in inaccurate or under-reporting, including the exaggeration of practice to be in accordance with perceived best practice. Using self-report also resulted in some areas having substantial amounts of missing data which may reduce generalisability. The strengths of the study were the cross sectional comparison of variable sites and the use of multiple data points in order to describe blood conservation and sampling practice. The study is the first to document paediatric and neonatal blood sampling values in order to inform and direct areas of practice improvement.

This research suggests adult patients in particular, and to some extent paediatrics could reduce the number of blood tests drawn. The results demonstrated the immediate pathology-associated direct costs of blood analysis; the accompanying disposable consumable costs and additional healthcare worker labour to draw the specimens also should be considered. If adult ICU pathology costs were decreased to the described neonatal estimates, this equates to a saving of >AUD$26million to Australian healthcare institutions annually. Additionally, the cumulative effect and costs due to iatrogenic anaemia in the critically ill are substantial and are well documented within adult ICU and healthcare institutions. If adult blood sampling volumes were reduced to the described neonatal volumes, this would result in over 6000 l less blood wasted annually due to testing and sampling. The direct and indirect costs associated with the processing, labour and consequences of potentially redundant diagnostic tests is continuing to place a significant encumbrance on strained healthcare systems.

The local CPGs in the ICUs were primarily focussed on safety, infection control and blood sampling process, rather than providing strategies to optimise blood conservation. Many blood conservation strategies were evidently being used within the critical care settings. However their implementation was not necessarily directed at the prevention of iatrogenic anaemia or the reduction in unnecessary blood sampling. This includes the use of non-invasive monitoring and point-of-care analysis. In comparison to the multiple, high profile, international CPG focussing on the reduction of healthcare-associated infections, little international effort has been made at translating the evidence available to support the reduction of iatrogenic anaemia to the critical care bedside.

5. Conclusions

Iatrogenic anaemia is a significant burden for critical care, and blood conservation strategies decrease its prevalence. This study

<table>
<thead>
<tr>
<th>Table 4: Blood conservation strategies from ICUs in local clinical practice guidelines and in practice.</th>
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<tbody>
<tr>
<td>Clinical practice guidelines</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>Neonatal ICU</td>
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<td>Adult ICU</td>
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described current practice of blood sampling and blood conservation in three critical care units in Australia. It has described the variation between these critical care areas in sampling frequency and volume, which are not adequately supported by research. Critically ill adults appear to be at high risk for potentially unnecessary blood sampling and testing, in comparison to the neonatal population. The clinical practice guidelines in use at each of the study sites were not reflective of evidence-based practice and demonstrates the low priority this area of clinical practice has been relegated. Continued vigilance and effort are needed to increase awareness among clinicians and support their efforts to identify and eliminate sources of unnecessary blood loss, such as duplicate tests and the continuance of routine tests past medical need. Ensuring minimisation of unnecessary blood loss from excessive sampling by reducing frequency or volume is within the scope of nursing practice. It is the responsibility of researchers to help clinicians examine practice and provide evidence to drive decision making processes. Further implementation projects and research are needed to accelerate implementation of known effective blood conservation strategies within critical care environments.

Authors' contributions

All authors contributed the conception and design of the study. AU, SK, FC, DL and KN were involved in acquisition of data, or analysis and interpretation of data. All authors have participated in drafting the article and revising it critically for important intellectual content. All authors have approved the final article and acknowledge that all those entitled to authorship are listed as authors.

Acknowledgements

We would like to thank Centre for Health Practice Innovation, Menzies Health Institute Queensland and Griffith University for funding. We would like to acknowledge and thank the staff on the study units at the Royal Children’s Hospital Paediatric Intensive Care Unit, Royal Brisbane & Women’s Hospital Intensive Care Unit and Neonatal Intensive Care Unit. We would like to thank the local study coordinators: Irene Smith, Tara Williams, Anne Dawbney and Alyson Eastgate.

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