Is the Whole Greater than the Sum of Its Parts? The Implementation and Outcomes of a Whole Blood Program in Ecuador

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Research article

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Abstract

Background: Hemorrhagic shock is a major cause of mortality in low-and-middle-income countries (LMICs). Many institutions in LMICs lack the resources to adequately prescribe balanced resuscitation. This study aims to describe the implementation of a whole blood program in Latin America and to discuss the outcomes of the patients that received whole blood (WB).

Methods: We conducted a retrospective review of patients resuscitated with WB from 2013-2019. Five units of O+ WB were made available on a consistent basis for patients presenting in hemorrhagic shock. Variables collected included: gender, age, service treating the patient, units of WB administered, units of components administered, admission vital signs, admission hemoglobin, Shock Index, Revised Trauma Score (RTS) in trauma patients, intraoperative crystalloid (lactated ringers or normal saline) and colloid (5% human albumin) administration, symptoms of transfusion reaction, length-of-stay and in-hospital mortality.

Results: The sample includes a total of 101 patients, 57 of whom were trauma and acute care surgery (TACS) patients and 44 of whom were obstetrics and gynecology patients. No patients developed symptoms consistent with a transfusion reaction. Average shock index was 1.16 (±0.55). On average, patients received 1.66 (±0.80) units of whole blood. Overall mortality was 14/101 (13.86%) in the first 24 hours and 6/101 (5.94%) after 24 hours.

Conclusion: Implementing a WB protocol is achievable in LMICs. Whole blood allows for more efficient delivery of hemostatic resuscitation and is ideal for resource-restrained settings. To our knowledge, this is the first description of a whole blood program implemented in a civilian hospital in Latin America.

Background

Hemorrhagic shock kills an estimated 1.9 million people annually worldwide. Prompt management of hemorrhagic shock is essential. Several decades of experience have shown that extensive volume resuscitation with crystalloid can lead to an exacerbation of the “lethal triad,” of trauma-induced coagulopathy, hypothermia and acidosis; this phenomenon has been recently modified to include hypocalcemia, and therefore has been termed the “lethal diamond”. Evidence over the past decade suggest a more balanced resuscitation involving the replacement of lost blood with a 1:1:1 transfusion with red blood cells (RBCs), fresh frozen plasma (FFP) and platelets, mimicking the whole blood (WB) that is lost. Adherence to a 1:1:1 ratio, however, remains challenging even in high-resource settings. Many regions of the world do not have the blood bank capabilities to adequately supply and respond to the challenging demands for providing and sustaining a timely and efficient 1:1:1 resuscitation strategy. As such, there has been renewed interest in WB transfusion programs as an initial approach to damage control resuscitation.
Hospital Vicente Corral Moscoso (HVCM) is a public hospital located in Southern Ecuador and serves as a referral center for more than 2 million inhabitants\(^\text{13}\). A WB transfusion program was implemented in 2013 to address the issue of inadequate hemostatic resuscitation for patients in hemorrhagic shock. The aim of this paper is to describe the logistics of the implementation of a WB transfusion protocol at our institution, review the outcomes of exsanguinating trauma and acute care surgery, and obstetrics and gynecology populations who received WB and demonstrate the feasibility of a WB program in a low-and-middle-income country (LMIC).

**Methods**

We performed a mixed methods analysis of the WB resuscitation program at the HVCM. In order to document the details of the program, we interviewed members of the blood bank staff, hospital administration, and clinical leadership of the hospital who were instrumental in establishing the program. In order to characterize the safety profile of WB transfusions in our program, we identified patients who received WB transfusion from 2013-2019. The following datapoints were collected: sex, age, service treating the patient, units of WB administered, units of components administered, admission vital signs, admission hemoglobin, Shock Index, intraoperative crystalloid and colloid administration, length-of-stay and in-hospital mortality. In trauma patients, the revised trauma score (RTS) was calculated. We also collected information suggestive of symptomatic acute transfusion reactions including evidence of anaphylaxis, urticaria, rash, jaundice, hemoglobinuria and respiratory difficulty in the immediate post-transfusion period. No labs were sent post-transfusion to detect subclinical transfusion reactions. Post-transfusion laboratory values were not available on all patients.

We used SPSS v 26 for statistical analysis. This study was approved by the ethical committee of our institution, Hospital Vicente Corral Moscoso, and the academic committee of research of the University of Azuay Medicine Faculty.

**Results**

*Whole Blood Transfusion Protocol at the HVCM*

Five units of type O+ WB were made available daily and released to patients presenting in hemorrhagic shock, as determined by the attending surgeon taking care of the patient. There is often a delay of only a few minutes in starting a WB transfusion once it is ordered by a physician as five units are always set aside for patients presenting in shock. After the type O+ whole blood is dispensed, the blood bank then proceeds to type and cross the patient using Bio-Rad Saxo ID-Reader. Initial resuscitation was begun in the emergency department and extended into the operating room in most cases, or is begun in the operating room. Given the resource limitations, a warming device was not routinely available. The patient is transfused with WB until vital signs stabilized per the clinical judgment of the attending surgeon, or until the blood bank is depleted of WB, after which type-specific RBCs and FFP are dispensed according to their availability in the blood bank. The decision to transition from WB to component therapy is not based on laboratory parameters at our institution as point-of-care laboratory tests are not available at our
institution to allow for rapid decision making. The resuscitation strategy at the HVCM is largely based on clinical exam, as tools such as thrombo-elastography (TEG) used at some institutions to guide blood-based resuscitation are not available.

**Blood donation and collection:** Whole blood is obtained through voluntary unpaid donation or replacement donation. The blood must be collected in no more than 12 minutes with constant manual or mechanical activation so as to prevent the activation of the clotting cascade. The use of aseptic technique is paramount for quality control and prevention of bacterial contamination. The sterile material utilized in this process is disposable. The extraction bags are quadruple bags including CPD+SAG-Mannitol BUFFY-COAT. The filtration system is used only when the blood is fractionated into components.

**WB processing, storage and administration:** After a unit of blood is donated, it is quarantined for 8 hours during which time the ABO type and Rh (D) type are determined. The WB is screened for the following serologic markers according to World Health Organization (WHO) standards[^14]: Syphilis, Hepatitis C, Hepatitis B, and HIV. Plasma antibody titers are not measured prior to the transfusion of WB. If the blood tests negative for infectious diseases, it is stored at 4-6°C and available for 48 hours. As such, the product dispensed at the HVCM is considered cold, fresh whole blood.

Leukoreduction is not routinely performed on the units of WB due to the inability to preserve platelets. After 48 hours, if the blood is not utilized, it is fractionated into components. The red blood cells and frozen plasma are then made available for up 35-42 days and one year respectively. Platelets are not available to patients at the HVCM after fractionation. Given the relative scarcity of blood products in the region, the platelets obtained through fractionation are primarily sent to the local cancer hospital. The blood bank stock is then replaced by the aforementioned methods.

**Outcomes of Patients Managed with WB:**
<table>
<thead>
<tr>
<th>Variable</th>
<th>Trauma and Acute Care Surgery, n (%)</th>
<th>Obstetrics and Gynecology, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>≤18 5 (8.8)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td></td>
<td>19-39 32 (56.1)</td>
<td>39 (88.6)</td>
</tr>
<tr>
<td></td>
<td>40-64 15 (26.3)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td></td>
<td>≥65 5 (8.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 45 (78.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Female 12 (21.1)</td>
<td>44 (100.0)</td>
</tr>
<tr>
<td>Number of WB units transfused in the first 24 hours</td>
<td>1 26 (45.6)</td>
<td>22 (50.0)</td>
</tr>
<tr>
<td></td>
<td>2 23 (40.4)</td>
<td>20 (45.5)</td>
</tr>
<tr>
<td></td>
<td>3 6 (10.5)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td></td>
<td>4 1 (1.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>5 0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>6 1 (1.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Blood Type</td>
<td>A+ 0 (0)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td></td>
<td>AB+ 1 (1.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>O+ 56 (98.2)</td>
<td>42 (95.5)</td>
</tr>
</tbody>
</table>

*Table 1. Demographics of patients receiving whole blood transfusion (n=101)*

One-hundred-and-one TACS or obstetrics and gynecology patients were identified as receiving resuscitation with WB. Fifty-seven patients presented to the TACS service, and 44 patients presented to the obstetrics and gynecology service with an average shock index of 1.16 (±0.55) (Table 1). Admission vital signs and laboratory values can be found in Table 2.
<table>
<thead>
<tr>
<th></th>
<th>Trauma and Acute Care Surgery (SD)</th>
<th>Obstetrics and Gynecology (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>96.00 (±30.62)</td>
<td>99.59 (±23.68)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>69.11 (±22.98)</td>
<td>71.44 (±17.63)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>100.82 (±26.23)</td>
<td>102.50 (±23.91)</td>
</tr>
<tr>
<td>Respiratory rate (breaths per minute)</td>
<td>21.79 (±6.47)</td>
<td>20.84 (±3.62)</td>
</tr>
<tr>
<td>Temperature (Celsius)</td>
<td>35.88 (±1.72)</td>
<td>35.90 (±1.89)</td>
</tr>
<tr>
<td>Glasgow Coma Score (GCS)</td>
<td>11.32 (±4.85)</td>
<td>14.45 (±1.98)</td>
</tr>
<tr>
<td>Shock Index</td>
<td>1.16 (±0.53)</td>
<td>1.14 (±0.58)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.19 (±3.13)</td>
<td>8.85 (±2.43)</td>
</tr>
<tr>
<td>Crystalloid administered in the operating room (mL)</td>
<td>2203.13 (±1667.03)</td>
<td>1497.94 (±778.78)</td>
</tr>
<tr>
<td>Colloid administered in the operating room (mL)</td>
<td>483.28 (±426.88)</td>
<td>400.06 (±438.62)</td>
</tr>
<tr>
<td>Estimated blood loss (mL)</td>
<td>1856.40 (±1577.35)</td>
<td>1569.49 (±1115.63)</td>
</tr>
</tbody>
</table>

*Table 2. Admission vital signs and laboratory values of patients resuscitated with WB*

On average, TACS patients received 1.75 (SD ±0.93) units of WB, and obstetrics and gynecology patients received 1.55 (SD ±0.59) units of WB. An average of 1.91 (SD ± 2.68) and 1.74 (SD ± 1.91) units of pRBCs were administered to TACS and obstetrics and gynecology patients respectively after WB. An average of 1.30 (SD ±2.59) and 0.84 (SD ±1.86) units of FFP were administered to TACS and obstetrics and gynecology patients respectively after WB. Platelets, as previously mentioned, are rarely available at our institution.

**TACS Patients**

Of the 57 patients on the TACS service, 47 were trauma patients and 10 were emergency general surgery patients. Average RTS in the trauma population was 6.06 (±1.89) and average shock index was 1.18 (±1). Anatomic injury data was not available in order to calculate an injury severity score. Of the 47 trauma patients, 24 suffered penetrating trauma and 23 suffered blunt trauma. Hemorrhage was localized in the abdomen in twelve (25.53%) patients, thorax in eleven (23.40%) patients, extremities in ten (21.27%) patients, thorax and abdomen in six (12.76%) patients, face in two (4.26%) patients, pelvis in two (4.26%) patients, spine in one (2.13%) patient, and neck in one (2.13%) patient. In the case of two patients (4.26%), the cause of hemorrhage was unknown as these patients expired prior to complete assessment.
The remaining 10 patients were emergency general surgery patients. Whole blood transfusion was utilized for intraoperative bleeding in a patient undergoing a repair of a ruptured abdominal aortic aneurysm (n=3, 30.00%), an open necrosectomy for infected pancreatic necrosis (n=1, 10.00%), a bowel resection for ischemic gut (n=1, 10.00%), a repair of an iliac artery aneurysm (n=1, 10.00%), repair of the superficial femoral artery following the excision of a left groin mass (n=1, 10.00%), control of gallbladder fossa hemorrhage following a laparoscopic cholecystectomy (n=1, 10.00%), control of splenic hemorrhage following a splenic biopsy (n=1, 10.00%), and control of hypogastric artery bleeding following a spinal surgery (n=1, 10.00%).

**Obstetrics and Gynecology Patients**

Forty-four patients presented to the obstetrics and gynecology service, with an average shock index of 1.15 (±0.58). Thirty-six patients (81.81%) suffered post-partum hemorrhage either after cesarean section or vaginal delivery. Post-partum hemorrhage was found to be due to uterine atony in eighteen (50.00%) patients, placenta accreta spectrum or placenta previa in eight (22.22%) patients, a tear in the vaginal mucosa in four (11.11%) patients, retained products in three (8.33%) patients, and uterine rupture in two (5.56%) patients. One (2.78%) patient had bleeding from an extrauterine vessel following a cesarean section. The remaining eight patients suffered hemorrhage due to a non-pregnancy-related gynecologic emergency (18.18%) including incomplete abortion in three (37.50%) patients, ruptured ectopic pregnancy in two (25.0%) patients, fibroids in one (12.50%) patient, post-operative bleeding after a hysterectomy in one (12.50%) patient, and a hemorrhagic ovarian cyst in one (12.50%) patient. The only mortality in this cohort expired due to complications related to hemorrhage from HELLP syndrome.

Length-of-stay in the intensive care unit was 4.79 (SD±5.85) days in TACS patients, and 1.84 (SD±2.77) days in obstetrics and gynecology patients. Length of stay was 13.07 (SD±15.33) days in TACS patients, and 5.86 (SD±4.56) days in obstetrics and gynecology patients (Figure 1).

**Adverse Outcomes**

Importantly, none of the patients in our series developed symptomatology consistent with acute transfusion reaction. This includes anaphylaxis, urticaria, rash, jaundice, and hemoglobinuria per the records from both the nurse and the primary surgical team. No patients were diagnosed with TRALI or TACO in the medical record. However, many of these patients were critically ill, and therefore intubated in the perioperative period making the diagnosis challenging. The cause of death in one trauma patient who suffered massive hemorrhagic shock from a penetrating brachial artery injury was noted to be massive pulmonary edema in the setting of multisystem organ failure. This could be consistent with a diagnosis of TRALI.

Overall mortality was 13.86% in the first 24 hours and 5.94% after 24 hours. The shock index of patients who expired in the first 24 hours and after 24 hours was 1.42 (SD±0.64) and 1.29 (SD±0.48), respectively. By comparison, patients who survived had an average shock index of 1.09 (SD±0.53). Of those patients that expired in the first 24 hours after transfusion, the cause of death was attributed to hemorrhage in
eleven patients, three of whom arrested prior to any operative intervention, six of whom arrested in the operating room, and three of whom arrested shortly after surgery. The two remaining patients expired due to a subarachnoid hemorrhage and irreversible shock postoperatively. One death in the maternal population occurred due to hemorrhage in a patient presenting with HELLP syndrome, severe preeclampsia and intrauterine fetal demise. The patient was found to have evidence of severe coagulopathy and a hepatic hematoma after undergoing a cesarean-section; this patient unfortunately died before to operative intervention could be undertaken.

Of the six patients that expired after 24 hours, three suffered sepsis leading to multisystem organ failure. Two patients were declared brain dead and one patient suffered irreversible shock after blunt force trauma to the chest not responsive to treatment.

Discussion

The HVCM is at the forefront of using WB transfusion in patients with hemorrhagic shock in Latin America. Our experience shows that WB transfusion represents a feasible strategy for achieving hemostatic resuscitation in patients presenting in hemorrhagic shock, especially in LMICs. Through the implementation of a whole blood program, we were able to establish a program wherein patients would receive balanced resuscitation. Prior to the implementation of a WB program, patients were resuscitated with whatever components were available in the blood bank, which included a varying and often scarce number of pRBCs and FFP. Of the patients resuscitated with WB, we do not describe any symptoms consistent with transfusion reactions.

The use of WB is not a new concept. Prior to the adoption of component therapy in 1970s, resuscitation of patients in hemorrhagic shock with WB was commonplace. Component therapy was popularized for logistical reasons and was never proven to be as efficacious as WB in hemorrhagic shock. Recent literature from the military suggest that WB may have superior or equivalent outcomes when compared to component therapy. Two retrospective combat casualty analyses compared the use of fresh whole blood to component therapy. One study described a mortality benefit in the WB group at 24-hours and 30-days, while the other found no statistically significant difference in mortality, death due to exsanguination, MSOF or sepsis. Nessen et al. showed that, in the experience with Forward Surgical Teams (FSTs) in Afghanistan, use of FWB in addition to RBCs and FFP was associated with improved in-hospital survival compared to RBCs and FFP alone. Within the civilian sector, retrospective analyses suggest superiority or equivalence of WB with regards to mortality and overall blood product use. The only randomized control trial in trauma patients, to our knowledge, compared modified whole blood (leukoreduced whole blood with additional platelet transfusion) with component therapy. After excluding patients with traumatic brain injury, a decrease in the use of component therapy was demonstrated in patients that received modified whole blood.

A recent systematic review and metanalysis comparing whole blood to component therapy found no difference in 24-hour or 30-day mortality. Importantly, this study highlighted the heterogeneity in the
literature on WB transfusion in trauma. No study in this analysis was from the civilian setting in a LMIC. Whole blood has important implications in LMICs, of which our work is only scratching the surface.

Whole blood has both, financial and logistical benefits compared to component therapy. Whole blood has the advantage of being easy to store, transport and administer to patients. Its use is ideal for resource-constrained environments and pre-hospital settings, especially those that do not have ready access to all blood components, delivering the product that would otherwise require three bags in one bag. Moreover, when separated components are transfused, each unit contains an increased amount of anticoagulants and additives that contribute to a patient’s overall coagulopathy compared to one unit of whole blood. Spinella et al. found that, in patients receiving massive transfusion, those receiving component therapy received a median of 825ml of additional additives and anticoagulants compared to the group resuscitated with WB. Additionally, it has been asserted that the administration of WB may actually be safer for patients with respect to risk of transmission of blood-borne diseases, exposing them to one donor instead of multiple donors.

Access to transfusion therapy and the “blood drought” creates significant barriers to safe surgery in LMICs. The use of WB is a potentially beneficial in countries where keeping up with a 1:1:1 transfusion ratio is not feasible. In resource-limited settings such as our institution in Southern Ecuador, a WB program has allowed us to streamline the prompt and expeditious administration of all blood components at once thus ensuring higher concentrations of all primordial elements thereby securing hemostatic strategy for patients in hemorrhagic shock. Prior to the implementation of this program, there was no way to guarantee the availability of balanced hemostatic resuscitation to patients in hemorrhagic shock.

It must be noted that Cuenca, Ecuador provides a favorable setting for implementing a whole blood program as 80% population is type O+ and 2% is type O- per the blood bank records. In our sample, most patients had type O+ blood. Due to the previously mentioned government initiative to reduce maternal mortality, the blood type of the obstetrics and gynecology patients was known prior to transfusion so as to prevent the possibility of Rh incompatibility. The high incidence of type O+ blood in the population makes it easier to identify donors and makes it more likely that a patient receiving a type O+ transfusion will be receiving a transfusion from a donor with the same blood type. While this is an advantage to implementation in our setting, this may make the study less generalizable to other populations.

The context for transfusion of patients in our region is distinct from the literature that typically recommends transfusion at a hemoglobin of 7g/dL. Located at 2,560 meters above sea level, the average hemoglobin in this population is higher than at lower altitudes. A study of hemoglobin levels published in Colombia described the average hemoglobin of those living at 2,600 meters to range between 14.6-15.2g/dl in women and 16.8-17.4g/dl in men. As such, patients at our institution are often transfused at a higher threshold, typically 9-10g/dL.
The primary concern with the transfusion of group O whole blood to non-group O recipients involves the possibility of minor ABO mismatch. Minor mismatch occurs when the anti-A and anti-B antibodies found in group O WB cause hemolysis in a non-group O recipient. This is typically mitigated by transfusion of only type-specific whole blood or through the transfusion of whole units with lows titer of antibody (most commonly <256 by the saline dilution, immediate spin method), known as low-titer O whole blood (LTOWB) \(^\text{16}\). Numerous studies documented the safety of LTOWB, demonstrating that it does not increase risk of transfusion reaction \(^\text{15, 16, 20, 21, 29, 34, 35}\).

However, at the HVCM, anti-A and anti-B titers are not measured prior to administration of blood products. Data supporting the transfusion of untitered whole blood is scarce in the literature. In a safety analysis of the transfusion of stored whole blood, Yazer et. al found that there was no statistically significant difference in the amount of incompatible plasma transfused in a unit of whole blood compared to the amount of incompatible plasma transfused in any unit of platelets \((p=0.38)\) \(^\text{23}\). Additionally, while group AB is considered to be the “universal plasma donor,” the military permits group A plasma to be dispensed in emergencies as group A individuals do not tend to make high anti-B antibodies and B red cells express the B antigen at a low density \(^\text{36}\). Sperry et. al. recently demonstrated a rate of adverse events possibly related to transfusions of 2.2% in a description of AB plasma and A plasma with low-titer B antibodies administered to patients in hemorrhagic shock in the pre-hospital setting \(^\text{37}\). The blood types of the patients in our sample were predominantly type O+, with a smaller percentage of AB+ and A+ individuals. No group B individuals were in our sample. Severe intravascular hemolytic transfusion reactions caused by minor mismatch are a relatively rare phenomenon \(^\text{19}\) that may be difficult to detect in an otherwise critically ill patient, and may not have been detected with the \(n\) of 93 in our series. While, again, we do not report any patients that developed symptomatology that was recognized as a hemolytic transfusion reaction, hemolysis labs post-transfusion were not routinely ordered. Several studies have documented that whole blood does not have evidence of increased hemolytic reactions with administration of up to 4 units \(^\text{20, 21, 38}\).

This study is limited in that it is a single-institution and retrospective study. There are many challenges that come with conducting research in a resource-limited setting, many of which affected this study. While more patients were identified than described, many files were unable to be located in medical records. The documentation at the HVCM continues to be written by hand, and many of the patient charts in medical records are missed pages or written illegibly. It should also be noted that documentation prior to the implementation of the TACS service (2012) was scarce. As such, the ability to compare this sample with patients resuscitated during an earlier time period is limited. The paucity of data in the paper charts also limited our ability to reliably determine the vital signs and labs following transfusion.

**Conclusion**

In summary, our experience demonstrates that whole blood administration in patients presenting with hemorrhagic shock can be an achievable alternative therapy to 1:1:1 resuscitation especially in limited
resource settings, wherein access to all blood products may not be feasible. Further studies comparing whole blood to component therapy in this population are needed. To our knowledge, this is the first description of a whole blood program implemented in a civilian setting in Latin America.

List Of Abbreviations

- WB=Whole Blood
- LMIC=Low- and middle-income country
- DCR=Damage control resuscitation
- HVCM= Hospital Vicente Corral Moscoso
- RTS=Revised Trauma Score
- WHO=World Health Organization
- TACS=Trauma and Acute Care Surgery
- RBCs=Packed red blood cells
- FFP=Fresh frozen plasma
- TEG= Thrombo-elastography

Declarations

Ethics approval: This study was approved by the ethical committee of our institution, Hospital Vicente Corral Moscoso, and the academic committee of research of the University of Azuay Medicine Faculty.

Consent to Participate: This is a retrospective study. Patients cannot be easily identified given the information extracted from the charts.

Funding: The authors have no funding to declare.

Competing Interests: The authors have no competing interests to declare.

Availability of data: The data collection sheet is provided in the electronic supplementary material. All identifying patient information has been removed for privacy.

Author contributions

- Data collection, data analysis, manuscript writing: AH
- Data collection, manuscript writing: MGA
- Data collection, data analysis: JRN, DSA, SGPP
- Critical review of manuscript: NFL, RPA, HAS, LFC, CCA
- Critical review of manuscript, final approval of manuscript for submission: JCP, JCSM

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**Supplementary Files**

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