

ACUTE NORMOVOLEMIC ANEMIA: PHYSIOLOGICAL AND PRACTICAL CONCERNS

P. Van der Linden

Department of Anesthesiology, CHU Brugmann, Brussels, Belgium

ACUTE NORMOVOLEMIC ANEMIA:PHYSIOLOGICAL AND PRACTICAL CONCERNS (Abstract): The adequacy of a hemoglobin concentration in a given clinical situation depends on whether a sufficient amount of oxygen is carried to the tissues to meet metabolic requirements. Therefore, the decision to transfuse a given patient cannot be based only on the hemoglobin level. Rather, rigid adherence to an arbitrarily predefined transfusion threshold will result in the over-transfusion of some patients, but also in the under-transfusion of others. A better knowledge of the physiologic responses developed during acute isovolemic anemia and the clinical factors that can limit the ability of the organism to maintain adequate tissue oxygenation in these situations, will allow the clinician to better define the transfusion trigger for each patient. This paper reviews the physiological and clinical factors of acute isovolemic anemia and presents the therapeutic options available.

KEY WORDS: NORMOVOLEMIC ANEMIA, PHYSIOLOGY, OXYGEN CONSUMPTION, TRANSFUSION

Correspondence to: Prof. Philippe Van der Linden, MD, PhD; CHU Brugmann - HUDERF, 4 Place Van Gehuchten, B-1020 Brussels, Belgium. e-mail: philippe.vanderlinden@chu-brugmann.be*

INTRODUCTION

The adequacy of a hemoglobin concentration in a given clinical situation depends on whether a sufficient amount of oxygen is carried to the tissues to meet metabolic requirements. Therefore, the decision to transfuse a given patient cannot be based only on the hemoglobin level. Rather, rigid adherence to an arbitrarily predefined transfusion threshold will result in the over-transfusion of some patients, but also in the under-transfusion of others [1]. A better knowledge of the physiologic responses developed during acute isovolemic anemia and the clinical factors that can limit the ability of the organism to maintain adequate tissue oxygenation in these situations, will allow the clinician to better define the transfusion trigger for each patient.

PHYSIOLOGIC RESPONSE TO ACUTE ANEMIA

The maintenance of tissue oxygen delivery during an acute reduction in red blood cell concentration depends on both an increase in cardiac output and an increase in blood oxygen extraction [2]. These two mechanisms require the preservation of an ample circulating blood volume.

The cardiac output response

Cardiac output increases during isovolemic anemia and the extent of this response appears to be closely related to the decrease in hematocrit. The cardiac output response is usually due to an increase in stroke volume and, to some extent, an increase in heart rate [2]. The decrease in blood viscosity plays a fundamental role in the rise in stroke volume by increasing venous return and decreasing total peripheral vascular resistance. These changes in cardiac loading conditions lead to improved myocardial functioning and a direct enhancement of myocardial contractility has also been described [3]. The decrease in total peripheral

* received date: 21.05.2007
accepted date: 4.06.2007

vascular resistance results, essentially, from reduced blood viscosity, but may also be related to the decreased scavenging capacity of blood to inactivate nitric oxide [4]. The adequate cardiac output response to isovolemic anemia also appears to be dependent on the presence of an intact autonomic nervous system and alpha-adrenergic tone [2].

The oxygen extraction response

The aim of the second compensatory mechanism is to better match oxygen delivery to oxygen demand at the tissue level. This mechanism, which permits the extraction of blood oxygen to increase, involves physiologic alterations at both the systemic and the microcirculatory level.

At the systemic level:

Matching oxygen delivery to tissue oxygen demand requires the redistribution of blood flow to areas of high demand (e.g. the brain and heart) in order to more effectively utilize oxygen held in venous blood [5]. Several experimental studies have demonstrated that cerebral and coronary vasodilatation occurs during acute anemia; as a result, blood flow in these areas increases out of proportion to the rise in cardiac output. This exceptional increase in blood flow to the brain and heart occurs because these organs are „flow-dependent” tissues, in contrast to other organs (e.g. the splanchnic area, kidneys, and skin) that are „flow-independent” tissues. Flow-dependent organs extract most of the oxygen available, even under basal conditions, and are unable to increase oxygen extraction further to meet their metabolic requirements.

Coronary blood flow increases even more than cerebral blood flow as myocardial oxygen demand increases during anemia. When the hematocrit is reduced to 10-12%, myocardial oxygen consumption more than doubles [6]. Under these conditions, coronary vasodilatation is nearly maximal. When the hematocrit is below 10%, coronary blood flow can no longer match the increased myocardial oxygen demand and ischemia develops, resulting in cardiac failure. This has been demonstrated in experimental data showing a decrease in systemic oxygen uptake when hematocrit values are close to 10% [7].

Excess perfusion to the brain and heart occurs at the expense of „flow-independent” organs. Relative vasoconstriction occurs in some tissues so that renal, mesenteric, and hepatic blood flows are proportionately less than the total cardiac output response. This regional blood flow redistribution among organs is partly due to alpha-adrenergic stimulation, but is unaltered in the presence of β -adrenergic blockade [8].

At the microcirculatory level:

Several physiological adjustments contribute markedly to providing a more efficient utilization of the remaining oxygen in the blood [9]. The main effect of hemodilution on the microcirculation is an increase in red blood cell velocity that allows red blood cell flux in the capillaries to be maintained up to a systemic hematocrit of 20%. This increased flow velocity stimulates arterial vasomotion and provides a more homogeneous distribution of red cells in the capillary network [9]. By shortening the transit time, the increase in red blood cell velocity may also reduce the loss of oxygen before it reaches the capillaries and, thereby, improve oxygen transfer to the tissues.

An increase in the ratio of microcirculatory to systemic hematocrit has also been demonstrated [10]. This phenomenon is related to complex interactions between axially migrating red blood cells (the Fahraeus effect) and the heterogeneous nature of the microcirculatory network. Finally, changes in the dynamics of the hemoglobin molecule can result in more efficient tissue oxygen delivery in anemia.

Indeed, a right shift of the oxyhemoglobin dissociation curve - which enhances oxygen release at a constant oxygen tension - begins at a hemoglobin level of 9 g/dL and becomes more prominent when levels are <6.5 g/dL [11]. This phenomenon results from

increased synthesis of 2,3 diphosphoglycerate and appears with declining hemoglobin after 12 to 36 hours.

TOLERANCE AND CLINICAL LIMITS OF ANEMIA

Maintenance of adequate tissue oxygenation during acute isovolemic anemia depends on the physiologic adjustments (described above) that occur at the systemic and microcirculatory levels and result in increased blood flow and oxygen extraction. The relative contribution of these 2 mechanisms depends on the ability of the patient to recruit each of them. Several studies have demonstrated that both are already involved in the early stages of isovolemic anemia [12]. They allow the maintenance of tissue oxygen balance until the hematocrit falls to about 10-12%. Below this „critical” value, oxygen delivery can no longer match tissue oxygen demand and cellular hypoxia develops. The critical hemoglobin value could, therefore, be defined as „the value of hemoglobin below which oxygen uptake becomes delivery-dependent”.

Experimental studies have demonstrated the critical hemoglobin value to be approximately 4.0 g/dL [13]. Corresponding values are obviously difficult to obtain in man. In a study in healthy conscious volunteers, Weiskopf et al demonstrated that tissue oxygenation remains adequate during severe isovolemic hemodilution up to a hemoglobin value of 5 g/dL [14]. Van Woerkens et al studied a Jehovah’s Witness patient who died from extreme hemodilution and observed a critical hemoglobin value of 4 g/dL [15]. Tolerance to severe acute isovolemic hemodilution not only depends on the integrity of compensatory mechanisms, but also on the level of tissue oxygen demand. For a given cardiac output and oxygen extraction response, any increase in tissue oxygen demand requires a higher hemoglobin level and, therefore, will reduce the patient’s tolerance to hemodilution.

Table I
Factors altering the physiologic response to isovolemic anemia

Factors associated with decreased cardiac output response
Hypovolemia
Cardiac failure, negative inotropic agents (e.g. β -blocking agents)
Coronary artery disease (CAD)
Valvular disease
Factors associated with decreased O₂ extraction response
Acute respiratory distress syndrome (ARDS)
Sepsis
Systemic inflammatory response syndrome (SIRS)
Traumatic injury
Ischemia-reperfusion syndrome
Vasodilating drugs
Factors associated with altered gas exchange
ARDS
Chronic pulmonary obstructive disease
Factors associated with increased O₂ consumption
Fever
Pain, stress, anxiety
Sepsis, SIRS
Hyperventilation syndromes

Any factor altering either the cardiac output response and/or the oxygen extraction response will reduce the patient’s tolerance to acute anemia (Table 1). Maintenance of adequate volume replacement is of paramount importance. The cardiac output response to hemodilution may be reduced in the presence of altered myocardial contractility. Acute

administration of negative inotropic agents (e.g. β -blocking agents) results in a decreased cardiac output response during hemodilution [16]. Coronary artery disease (CAD) will obviously limit the tolerance of the heart to isovolemic hemodilution. As myocardial oxygen extraction is already nearing maximal during resting conditions, the maintenance of myocardial oxygen consumption depends essentially on the increase in coronary blood flow. Therefore, the coronary reserve (the ratio between maximal coronary blood flow and resting coronary blood flow) is significantly reduced during hemodilution, especially in CAD patients who already have decreased maximal coronary blood flow.

The lowest tolerable hematocrit in CAD patients is not known, but experimental data on animals with extrinsically applied coronary stenosis has demonstrated a significant increase in the critical hematocrit level to 17-18% [17]. Even if CAD patients can tolerate some degree of hemodilution intraoperatively, they will require a higher hematocrit in the early postoperative period to meet increased tissue, and especially cardiac, oxygen demand. Cardiovascular disease patients with a lower preoperative hematocrit have an increased risk of death when compared to noncardiovascular disease patients with the same preoperative hematocrit [18].

In patients with no evidence of cardiovascular disease, age alone does not seem to be a major factor in determining tolerance to anemia, although compensatory mechanisms to an acute reduction in blood oxygen content may be less efficient [19].

Controlled hypotension is frequently used during surgical procedures to decrease perioperative blood loss. However, the use of vasodilating agents and, in particular, alpha-blocking agents, may interfere with the normal regional redistribution of blood flow during hemodilution. Experimental studies have demonstrated impaired renal and splanchnic tissue oxygenation when controlled hypotension is superimposed on isovolemic hemodilution [20].

Respiratory insufficiency also limits the physiologic adjustment to acute anemia. On the one hand, altered arterial oxygenation contributes to the decrease in oxygen-carrying capacities of the blood. On the other hand, hemodilution could have a deleterious effect on pulmonary gas exchange, possibly through attenuation of hypoxic pulmonary vasoconstriction [21]. Although the optimal hematocrit during respiratory insufficiency is not known, patients with chronic respiratory failure develop polycythemia in an attempt to maintain adequate tissue oxygen delivery.

During a critical illness, most of the compensatory mechanisms for anemia are reduced by the presence of hypovolemia, hypoxemia, depressed myocardial function, and/or altered tissue oxygen extraction capabilities. In addition, tissue oxygen demand is often increased in these situations due to fever, pain, stress, and increased respiratory effort. Therefore, it is not surprising that anemia is associated with an increased risk of morbidity and mortality in critically ill patients, especially in those with cardiovascular disease. However, there is no evidence in the literature that the use of a more liberal transfusion strategy in this „at risk” population is associated with better outcomes [22].

THERAPEUTIC OPTIONS DURING CRITICAL HEMODILUTION

Maximizing cardiac output

The efficacy of mechanisms to preserve tissue oxygen delivery when the oxygen-carrying capacity of the blood is reduced depends primarily on maintenance of an adequate blood volume. This is especially true for the cardiac output response to hemodilution. Indeed, hypovolemia will blunt the effects of decreased blood viscosity on venous return [23]. Crystalloid solutions alone may be insufficient because of rapid extravascular redistribution. Synthetic colloids may thus be required. Depending on the size and structure of the macromolecules and their actual concentration in the blood, any solution containing artificial

colloids may increase plasma viscosity. An increase in plasma viscosity elicited by exchange of whole blood for colloid solutions may jeopardize microvascular perfusion and tissue oxygenation. However, the impact of plasma viscosity on the rheological properties of whole blood is completely offset by the concomitant reduction in hematocrit [24]. The critical hemoglobin level does not appear to be influenced by the type of synthetic colloid (e.g. 6% hydroxyethyl starch 200/05 or 3% modified fluid gelatin) [13].

Increasing oxygen content

The inspired fraction of oxygen (FiO_2) may also influence the critical hemoglobin level since dissolved oxygen in plasma increases markedly during hemodilution [24]. However, hyperoxemia reduces the cardiac output response occurring during isovolemic anemia and partially reverses the decrease in systemic vascular resistance. Nevertheless, tissue oxygenation appears to be improved under these conditions, as the microcirculatory changes induced by hyperoxemia (arteriolar vasoconstriction mediated locally by the arachidonic acid metabolic pathway) may be at least partially blunted by hemodilution-induced vasodilatation [6]. A recent experimental study demonstrated that hyperoxic ventilation increases short-term survival in anesthetized pigs undergoing critical hemodilution [26]. However, high FiO_2 (50%-100%) can be administered only for short periods of time. Indeed, increased FiO_2 for long periods of time induces ongoing free radical formation in the lungs, with subsequent lung tissue damage [27].

DECREASING METABOLIC RATE

Moderate hypothermia

Deliberate mild hypothermia has been used in the per- and postoperative management of severe anemic patients [28]. Moderate hypothermia decreases tissue oxygen demand, but also increases the amount of oxygen dissolved in plasma and improves tissue affinity for oxygen.

Table II
Possible effects of anesthetic agents on the physiologic response to hemodilution

Effects on the cardiac output response
Alteration in cardiac loading conditions
Negative inotropic properties
Depressed autonomic nervous system activity
Effects on the O₂ extraction response
Vasodilation
Depressed autonomic nervous system activity
Effects on gas exchange
Decreased functional residual capacity
Effects on tissue oxygen demand
Relief of pain, stress, anxiety
Decreased myocardial oxygen demand (negative inotropic and chronotropic effect)
Decreased muscular activity

A target core temperature of 30-32°C is usually chosen because it decreases oxygen consumption by 48% below basal level and increases the amount of dissolved oxygen in the plasma by 10%, without significant cardiac side effects [28]. However, hypothermia also has negative effects on tissue oxygenation. Hypothermia is associated with a leftward shift of the

oxyhemoglobin dissociation curve, increasing hemoglobin affinity for oxygen. Therefore, the net effect of hypothermia on the critical level of hemodilution remains to be determined.

Anesthesia - sedation

Anesthesia (or sedation) and ventilatory support have been used in severely anemic patients in an attempt to reduce their oxygen consumption. However, anesthesia can alter the physiologic adjustments to isovolemic hemodilution at different levels (Table 2). Because most anesthetic agents depress the cardiovascular and autonomic nervous system in a dose-dependent manner, it could be hypothesized that the most striking effect of anesthesia would be a decreased cardiac output response to isovolemic hemodilution. This hypothesis has been confirmed [29]. Since anesthesia decreases the cardiac output response to isovolemic hemodilution - but may also decrease tissue oxygen demand - the effects of anesthesia on a patient's tolerance to severe anemia („critical” hemoglobin level) depends on the balance between these two effects.

THE TRANSFUSION TRIGGER

The adequacy of any hemoglobin concentration in a given clinical situation depends on whether a sufficient amount of oxygen is being carried to the tissues to meet their oxygen requirements. Clinical signs of inadequate tissue oxygenation during anemia (e.g. tachycardia, postural hypotension, dizziness etc.) are very sensitive, but non-specific. Moreover, they are usually absent in sedated or anesthetized patients. In critically ill patients, the mixed venous oxygen saturation (SvO₂) is frequently used to detect the development of an imbalance between oxygen supply and uptake. In a Jehovah's Witness patient dying from extreme hemodilution, the critical hemoglobin level was reached at a SvO₂ value of 56% and an oxygen extraction ratio of 44% [15]. Several clinical observations tend to indicate that the SvO₂ (or the oxygen extraction ratio) might be a reliable physiologic guide to transfusion [30,31].

Only a few well-conducted studies have evaluated the efficacy of transfusion strategies based on hemoglobin level. Carson et al [32] reviewed 10 randomized trials comparing the effects of a „liberal” vs „restrictive” transfusion strategy - based on a specified hemoglobin (or hematocrit) concentration - on short-term out-come (1780 patients). Applying a restrictive strategy significantly reduced the likelihood of patients being transfused, as well as the number of blood units transfused, without affecting patient outcome. It must be emphasized, however, that none of these studies evaluated very anemic patients (e.g. a hemoglobin level < 7.0 g/dL). Moreover, the major problem with the studies assessing the effectiveness of different transfusion strategies is that they also evaluate the efficacy of red blood cell transfusion. All the studies evaluating the efficacy of transfusion strategies were performed before the implementation of universal leukoreduction that, by itself, might impact the mortality and morbidity associated with blood transfusion [33]. The real efficacy of allogeneic red blood cells that are older than several days to improve oxygen delivery at the tissue level remains to be debated.

Therefore, the current literature suggests that it is unlikely that any level of hemoglobin can be used as a universal threshold for transfusion. *The decision to transfuse an individual patient depends on medical judgement, taking into account not only the hemoglobin concentration, but also the physical status of the patient (his/her physiological reserve), the clinical conditions (ongoing blood loss, sepsis, sedation etc), and available monitoring.*

CONCLUSION

An acute decrease in blood oxygen carrying capacities during anemia elicits physiologic adjustments at both the systemic and microcirculatory level, resulting in an increase in both cardiac output and tissue oxygen extraction. In physiologic situations, these are very efficient as they allow maintenance of tissue oxygen delivery up to a systemic hematocrit of 10-15% during resting conditions. In pathophysiologic situations, tolerance to acute anemia depends on the body's ability to recruit each mechanism and the level of tissue oxygen demand. In any case, maintenance of adequate volume replacement is of paramount importance. In the perioperative setting, SvO₂, which is usually used to detect the development of an imbalance between oxygen supply and uptake, might be a reliable physiologic guide to transfusion. The decision to transfuse a given patient should not be based on hemoglobin level only.

REFERENCES

1. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion Medicine. Blood transfusion. *N Engl J Med.* 1999; 340(6): 438-447.
2. Chapler CK, Cain CM. The physiologic reserve in oxygen carrying capacity: studies in experimental hemodilution. *Can J Physiol Pharmacol.* 1986; 64: 7-12.
3. Habler OP, Kleen MS, Podtschaske AH, Hutter JW, Tiede M, Kemming GI, Welte MV, Corso CO, Messmer KF. The effect of acute normovolemic hemodilution (ANH) on myocardial contractility in anesthetized dogs. *Anesth Analg.* 1996; 83(3): 451-458.
4. Doss DN, Estafanous FG, Ferrario CM, Brum JM, Murray PA. Mechanism of systemic vasodilation during normovolemic hemodilution. *Anesth Analg.* 1995; 81(1): 30-34.
5. Tuman KJ. Tissue oxygen delivery: the physiology of anemia. *Anesthesiol Clin North Am.* 1990; 8: 451-469.
6. Habler OP, Kleen MS, Hutter JW, Podtschaske AH, Tiede M, Kemming GI, Welte MV, Corso CO, Batra S, Keipert PE, Faithfull NS, Messmer KF. Effects of hyperoxic ventilation on hemodilution induced changes in anesthetized dogs. *Transfusion.* 1998; 38(2): 135-144.
7. Cain SM. Oxygen delivery and uptake in dogs during anemic and hypoxic hypoxia. *Appl Physiol.* 1977; 42: 228-234.
8. Crystal GJ, Ruiz JR, Rooney MW, Salem MR. Regional hemodynamics, and oxygen supply during isovolemic hemodilution in the absence and the presence of high-grade beta-adrenergic blockade. *J Cardiothorac Vasc Anesth.* 1988; 2(6): 772-780.
9. Messmer K. Blood rheology factors and capillary blood flow. In: Gutierrez G, Vincent J-L, editors. *Tissue oxygen-utiUzation.* Berlin, Heidelberg, New-York: Springer-Verlag; 1991. p. 103-113.
10. Lindbom L, Mirhashemi S, Intaglietta M, Arfors K-E. Increase in capillary blood flow relative to hematocrit in rabbit skeletal muscle following acute normovolemic anemia. *Acta Physiol Scand.* 1988; 134(4): 503-512.
11. Sibbald WJ, Doig GS, Morisaki H. Role of RBC transfusion therapy in sepsis. In: Sibbald WJ, Vincent J-L, editors. *Clinical trials for the treatment of sepsis.* Berlin, Heidelberg, New York: Springer Verlag; 1995. p. 191-206.
12. Spahn DR, Leone BJ, Reves JG, Pasch T. Cardiovascular and coronary physiology of acute isovolemic hemodilution: a review of nonoxygen-carrying and oxygen-carrying solutions. *Anesth Analg.* 1994; 78(5): 1000-1021.
13. Van der Linden P, De Groot F, Mathieu N, et al. Critical haemoglobin concentration in anaesthetized dogs: comparison of two plasma substitutes. *Br J Anaesth.* 1998; 81(4): 556-562.
14. Weiskopf RB, Viele MK, Feiner J, Kelley S, Lieberman J, Noorani M, Leung JM, Fisher DM, Murray WR, Toy P, Moore MA. Human cardiovascular and metabolic response to acute, severe isovolemic anemia. *JAMA.* 1998; 279(3): 217-221.
15. van Woerkens ECSM, Trouwborst A, van Lanschot JJB. Profound hemodilution: what is the critical level of hemodilution at which oxygen delivery-dependent oxygen consumption starts in an anesthetized human? *Anesth Analg.* 1992; 75 : 818-821.
16. Lieberman JA, Weiskopf RB, Kelley SD, Feiner J, Noorani M, Leung J, Toy P, Viele M. Critical oxygen delivery in conscious humans is less than 7.3 ml O₂.kg.min⁻¹. *Anesthesiology.* 2000; 92(2): 407-413.
17. Levy PS, Kim SJ, Eckel PK, Chavez R, Ismail EF, Gould SA, Ramez Salem M, Crystal GJ. Limit to cardiac compensation during acute isovolemic hemodilution: influence of coronary stenosis. *Am J Physiol.* 1993; 265(1 Pt 2): H340-H349.

18. Carson JL, Duff A, Poses RM, Berlin JA, Spence RK, Trout R, Noveck H, Strom BL. Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. *Lancet*. 1996; 348: 1055-1060.
19. Janvier, G. and Annat, G. Are there limits to haemodilution? *Ann Fr Anesth Reanim*. 1995; 14(Suppl I): 9-20.
20. Crystal GJ, Rooney MW, Ramez Salem M. Regional hemodynamics and oxygen supply during isovolemic hemodilution alone or in combination with adenosine-induced controlled hypotension. *Anesth Analg*. 1988; 67: 211-218.
21. Deems S, Bishop MJ, Alberts MK. Effect of anemia on intrapulmonary shunt during atelectasis in rabbits. *J Appl Physiol*. 1995; 79: 1951-1957.
22. Hebert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E. A multicenter randomized controlled clinical trial of transfusion requirements in critical Care. *N Engl J Med*. 1999; 340(13): 409-417.
23. Richardson TQ, Guyton AC. Effects of polycythemia and anemia on cardiac output and other circulatory factors. *Am J Physiol*. 1959; 197: 1167-1170.
24. Bruckner UB, Messmer K. Blood rheology and systemic oxygen transport. *Biorheology*. 1990; 27(6): 903-912.
25. Habler OP, Messmer K. Hyperoxemia in extreme hemodilution. *Br J Anaesth* 1998; 81: 79-82.
26. Meier J, Kemming GJ, Kisch-Wedel H. Hyperoxic ventilation reduces 6-hours mortality at the critical hemoglobin concentration. *Anesthesiology*. 2004; 100: 70-76.
27. Klein J. Normobaric pulmonary oxygen toxicity. *Anesth Analg*. 1990; 70: 195-207.
28. Culkin Mann M, Votto J, Kambe J, Mc Namee MJ. Management of the severely anemic patient who refuses transfusion; lessons learned during the care of a Jehovah's witness. *Ann Intern Med*. 1992; 117: 1042-1048.
29. Ickx B, Rigolet M, Van der Linden P. Cardiovascular and metabolic response to acute normovolemic anemia: effects of anesthesia. *Anesthesiology*. 2000; 93: 1011-1016.
30. Fontana JL, Welborn L, Mongan PD, Sturm P, Martin G, Bünger R. Oxygen consumption and cardiovascular function in children during profound intraoperative normovolemic hemodilution. *Anesth Analg*. 1995; 80(2): 219-225.
31. Paone G, Silverman NA. The paradox of on-bypass transfusion thresholds in blood conservation. *Circulation*. 1997; 96 (suppl II): II 205-II 209.
32. Carson JL, Hill S, Carless P, Hebert P, Henry D. Transfusion triggers: a systematic review of the literature. *Transfus Med Rev*. 2002; 16(3): 187-199.
33. Hebert PC, Fergusson D, Blajchman MA, Wells GA, Kmetz A, Coyle D, Heddle N, Germain M, Goldman M, Toye B, Schweitzer I, vanWalraven C, Devine D, Sher GD; Leukoreduction Study Investigators. Clinical outcomes following institution of the Canadian universal leukoreduction program for red blood cell transfusions. *JAMA*. 2003; 289(15): 1941-1949.