Review Article

Implications and Considerations for Analysis of Inflammatory Markers in the Red Cell Concentrates of Borderline Diabetic Blood Donors

ABSTRACT

Storage lesions of blood products intended for transfusion have been shown to give rise to the accumulation of proinflammatory cytokines, which are known to cause transfusion associated adverse events and immunomodulatory effects. Regulations which determine the eligibility of blood donors do not take into consideration metabolic disorders such as Type 2 Diabetes Mellitus (T2DM), unless complications arise from said disorder. However, the increased levels of cytokines in T2DM classifies this condition as an inflammatory condition. This outcome dictates the need to investigate whether detected levels of pro-inflammatory cytokines due to inflammation combined with those present in red cell concentrates as a result of storage lesions may lead to undesirable outcomes in recipients who already have underlying inflammatory conditions. Due to the largely unavailable information on the subject, a few important considerations for undertaking such investigations have been summarised.

Keywords: Adverse Transfusion Reactions, Cytokines, Inflammation, Red Cell Concentrate Storage, Blood Donors, Inflammatory disorders.

Introduction

Inflammation is an innate mechanism of the body which occurs in response to external factors such as tissue injury, allergy and infection(1). This mechanism is regulated by small cell-derived proteins termed cytokines(2). Cytokines regulate inflammation(3) and have both a synergistic and antagonistic effect on multiple mechanisms, which in turn may eventually lead to either beneficial or adverse outcomes(4,5). Dysregulation of cytokines causes chronic inflammation and this regulatory failure is often associated with an increase in age(6). Furthermore, cytokines are now recognised as being inflammatory markers of both auto-immune and auto-inflammatory disorders such as Type 2 Diabetes Mellitus (T2DM) and studies have shown that cytokine levels are already increased at the pre-diabetic (borderline) stage(7–9). In blood transfusion, red blood cells have been described as being a reservoir of cytokines with an active role in cytokine signalling which culminates in an inflammatory response(10). This review aims to highlight a few issues encountered during a preliminary trial study (data not published) to determine the level of a selected group of cytokines in blood donors with known or undiagnosed T2DM. This study was derived from the combination of two concepts. The first one being that dysregulation of pro-inflammatory and anti-inflammatory cytokines plays a role in the pathogenesis of a variety of diseases, especially inflammatory diseases (5,11), and the second one being based on the knowledge that accumulation of cytokines is seen in stored blood products due to storage lesions (10,12,13) which presence are mainly caused by aggregates and biochemical debris and in turn contribute to immunomodulatoty factors responsible for Transfusion-associated Adverse Reactions (TAAR)(14). These two concepts have led to the idea that RCCs from blood donors with an inflammatory disorder would have higher cytokine levels, since although methods such as leucodepletion reduce the formation of cytokines during storage, to date there is no method to manage pre-existing cytokines present in the donated blood. In brief, the preliminary study evaluated the concentrations of pro-inflammatory cytokines, interleukin (IL)-1 beta, IL-

6 and Tumour Necrotic Factor (TNF)-alpha, in packed red cells collected from voluntary blood donors between the ages of 55-68 years who were then categorised into non-diabetic, borderline diabetic and diabetic blood donors according to their blood glucose at pre-donation stage. Since regulations that govern the eligibility of blood donors do not prevent individuals with diabetes (or any other inflammatory disorder) from donating blood unless the use of insulin is required or other underlying conditions are present, the scope of the study was to elucidate the effects of inflammatory diseases in combination with TAARs, in particular Febrile Non-Haemolytic Transfusion Reactions and Transfusion-Related Acute Lung Injury, with the latter considered the adverse reaction with the highest rate of mortality (14,15). For example, IL-6 and TNF-alpha are two of the major cytokines associated with these adverse reactions and studies have reported an increase of these cytokine levels in red cell concentrates (RCCs) due to storage lesions(16). Based on such information there is a lack of knowledge on what effect such donations may have in recipients who already have elevated cytokine levels such as in the cases of critically-ill, oncology or neonatal patients, so such an endeavour would shed light on potential clinical implications(17).

Role of Cytokines

Cytokines are known to vary with sex and age and studies have identified that changes may occur at different stages in women and men. For example, menopause is characterised by higher cytokine levels. Commonly reasons for the age-related increase in cytokine levels include underlying comorbidities or the decline in serum dehydroepiandrosterone with increasing age. Obesity is another factor that affects cytokine levels(18). Cytokine genes have been found to be highly polymorphic, with the genotype impacting cytokine production(19). This makes determining biologically significant differences challenging(11).

Cytokine levels are easily affected by a variety of conditions ranging from both technical and methodological factors to others which are physiological, such as genetic variability(19) and even ones which are environmental in nature. On this note, it is important to highlight that although the blood donors selected for this trial met the eligible criteria to donate blood, there is still the possibility that the donor could have an undiagnosed disease or be suffering from an asymptomatic infection. An important factor not taken into consideration when recruiting subjects was whether these were smokers or non-smokers – studies have shown an elevated level of IL-6 in smokers(20). Having said that, it is not feasible to obtain a full medical history of the participants, however a set of questions can aid in preventing additional factors from confounding the results. Such questions could include the participants' weight and height to determine the body mass index, and when was the last intake of food and drink (other than water) as this would impact the glucose level reading for a provisional categorisation. To aid such categorisation, self-reporting T2DM participants should provide details related to the stage of diabetes, when this was diagnosed and if glycaemic control is attained via drugs or diet. In addition the participant could be asked to tick a list of conditions such as respiratory conditions, heart conditions, arthritis, and inflammatory bowel disease, which are known to affect cytokine levels but do not cause donor-deferral.

The disadvantage of recruiting participants from blood donation centres is the voluntary blood donation setting *per se*. One such factor is not being able to restrict sampling to a specific time of the day, as studies postulate variations of cytokine levels with the diurnal and nocturnal cycle(21). In addition, ethical implications should also be considered to minimise invasive sampling to determine the glucose level. From a list of glucose monitoring tests recommended by the American Diabetes Association, the blood glucose by Point-of-Care-Testing, although less accurate, resulted as being the least invasive. However, cut-off values should be reconsidered when used for such studies, since blood donors are encouraged to partake of a light snack before donating blood and not enough time (calculated to be two hours) may have elapsed to allow the glucose levels to stabilise once again. Recruiting blood donors for this study with known T2DM or prediabetes was a major issue and the main limiting factor in terms of sample size. Such individuals may not full classify as eligible blood donors or a possible misconception of the general public could exist where the idea that people suffering from such conditions may not donate blood. Another possibility, is that the recommendation that donors with T2DM should be aware of fluctuations in blood glucose post blood donation could also deter such an enterprise.

Level of cytokine concentrations

Considering that the scope of the preliminary study was to monitor the cytokine concentration in RCCs, on hindsight, the samples used would have been best collected directly from the RCC units instead of donor blood in ethylenediaminetetraacetic acid at the pre-donation stage. Ideally both scenarios should be considered for a paired analysis of both states, namely before and during storage. Blood is not transfused as whole blood and needs to undergo several processes before it can be administered to the patient. Such processes may also have an impact on cytokine levels, as it is well known that storage lesions may arise from such manipulations. Although RBCs are known reservoirs of cytokines(10,12,22), the quantification of such cytokines is still in its early stages, limiting the amount of knowledge available, as is the unavailable standard range of cytokines in healthy individuals(11).

Cytokine-related studies are generally carried out to determine the difference in cytokine levels associated with one particular disorder or co-morbidity and a normal cohort. The relationship between inflammatory disorders and transfusion implications is largely underestimated and no studies have, to date, been undertaken in this regard. Chronic inflammation has been shown to play an important yet not defined role in the pathophysiology of T2DM. Considering the increased rate of pre diabetes and T2DM diagnosed worldwide, not to mention other inflammatory disorders, there is the need to determine the consequences when such blood is transfused to recipients with already elevated cytokine levels, in particular cancer and paediatric patients, in whom immune suppression and excess inflammation are known to produce adverse outcomes(23).

Conclusion

The remarks and recommendation mentioned in this review aim to set the grounds for a novel area of research which may lead to the introduction of a number of patient-targeted quality parameters for blood components, in order to mitigate transfusion adverse events in those recipients most at risk.

CONSENT

All participants where pseudo anonymous and couldn't be identified. Informed consent from the participants was granted during recruitment.

ETHICAL APPROVAL

Ethical approval was granted by the University of Malta Ethics Research Committee (12/2017).

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

T2DM - Type 2 Diabetes Mellitus, RCCs - Red Cell Concentrates, IL – Interleukin, TNF - Tumour Necrotic Factor, TAAR – Transfusion-associated adverse reactions