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Haemolysis is a concerning bias in some ozone therapy approaches

To the Editor,

During the oxygen-ozone major autohaemotherapy (O_2-O_3-MAHT) the cellular component of the autologous peripheral blood undergoes stressing mechanical forces, which usually cause the production of cell microparticles and haemolysis, although without a real concerning impact on patients' safety if protocols recommended by the Italian Scientific Society of Oxygen-Ozone Therapy (SIOOT) are considered [1,2]. Generally, to estimate how much red blood cells (RBCs) might be disrupted (haemolysis) under two different hypothetical scenarios involving different needles and flow rates, we have to consider mechanical stress factors such as shear stress, needle size, and flow rate, including positive pressure devices, used, for example, in the EBOO techniques, which are not recommended by SIOOT at all [3].

Fundamentally, the best clinical practice to collect and transfer autologous peripheral blood treated with oxygen-ozone, considers phthalate-free disposable plastic bags for haemo-transfusion ($SANO_3$) [1] and relatively large needles, such as an 18-gauge (G) needle, which has an internal diameter of about 1.27 mm and allows a reasonably high flow rate, thus minimizing mechanical stress on RBCs. At 80 drops per minute (where one drop is roughly 0.05 mL), this translates to approximately 4 mL/min. With a larger lumen size (1.27 mm), the flow is relatively smooth, and the RBCs are subjected to less mechanical stress. Haemolysis in such a scenario is typically very low, quite null. In this circumstance, given the relatively moderate flow rate and large needle size, RBC disruption is expected to be minimal. In clinical studies, transfusions with 18G needles show negligible haemolysis levels, often measured as <0.1 % increase in free haemoglobin, which is clinically insignificant and adverse effects are very rare [4].

When the extracorporeal blood flow is subjected to a positive pressure, the amount of RBC undergoing damage is undoubtedly higher. This can occur also if the whole peripheral blood enters a glass bottle for the negative pressure caused by the lower air content in the bottle than outside.

In this circumstance, haemolytic foam can occur (Fig. 1). The foam formation in glass bottles during blood collection or transfusion can indeed be a cause for concern, as excessive foam can lead to red blood cell (RBC) damage and haemolysis. Foam is generated when air mixes with the blood, especially when blood enters the bottle rapidly and hits the hard, non-flexible glass surface. Then, turbulence occurs as the blood flows into the bottle, creating air bubbles. Moreover, shaking or agitation exacerbates the mixing of air and blood, trapping air bubbles in the blood. When blood flows into a glass bottle, the rigid surface does not absorb any of the impact, and this can increase the turbulence and mixing with air, leading to foam formation. Obviously, the formation of foam is problematic for several reasons. A first reason is due to mechanical stress: the air bubbles in foam create additional shear forces on RBCs. When RBCs come into contact with these air bubbles and are subjected to surface tension, they can rupture, leading to haemolysis. A second reason is due to surface tension effects: when RBCs encounter the air–liquid interfaces in foam, they are stretched and stressed at these boundaries, particularly if the inner surface of glass shows micro- and nano-fractures. This can cause the cell membrane to rupture, releasing haemoglobin into the plasma and contributing to enhance haemolysis. A third reason is the increased exposure to oxygen: while blood needs oxygen, excessive exposure to air due to foaming can oxidize haemoglobin and cause oxidative stress on RBCs, potentially leading to cell damage.

Actually, in glass bottles, where foam formation is more likely due to the rigid walls and potential for turbulence, it is particularly frequent phenomena of localized haemolysis, where RBCs are at higher risk of disruption when passing through or around foam, leading to localized haemolysis, where the RBCs near the foam break down more readily but also of an increased haemolysis risk. Foam increases the surface area of air contact with blood, leading to a higher chance of RBC rupture. The haemolysis level can rise significantly if excessive foam persists, potentially leading to 1–3 % haemolysis or more depending on the extent of the turbulence and foam.

Such a percentage can be even achieved by using, for example, a 22-G needle. Smaller needles (like 22-G), particularly under high pressures, can cause more RBC destruction. Haemolysis levels can increase significantly in such conditions. Some studies report up to 1–2 % free haemoglobin increase in cases with high-pressure infusion through smaller needles [5].

In contrast, plastic bags are designed to minimize foam formation. They are made of soft and flexible material. The flexible surface absorbs much of the force of the incoming blood, leading to less turbulence and air mixing. And furthermore, they cause minimized air exposure. Blood collection systems using plastic bags are usually designed to reduce air contact, limiting the formation of foam and reducing haemolysis risk [1,2].

Addressing the concern of haemolysis is not an exquisite topic of academic erudition. Haemolysis is particularly burdensome for many plasma-related mechanisms, including coagulation and platelets activation.

Free haemoglobin and iron are particularly concerning for thrombotic complications [6,7]. Extracellular free haeme behaves as an alarmin, enabled to trigger pro-oxidative and pro-inflammatory responses and subsequently activating platelets, innate immune cells and endothelia, impairing the coagulation cascade and causing micro-thrombotic events [7].

A 1–2 % increase in free haemoglobin due to haemolysis, particularly if linking to the von Willebrand factor (vWF) can contribute to a prothrombotic state by scavenging nitric oxide, damaging the endothelium, promoting hypercoagulability, and activating the complement

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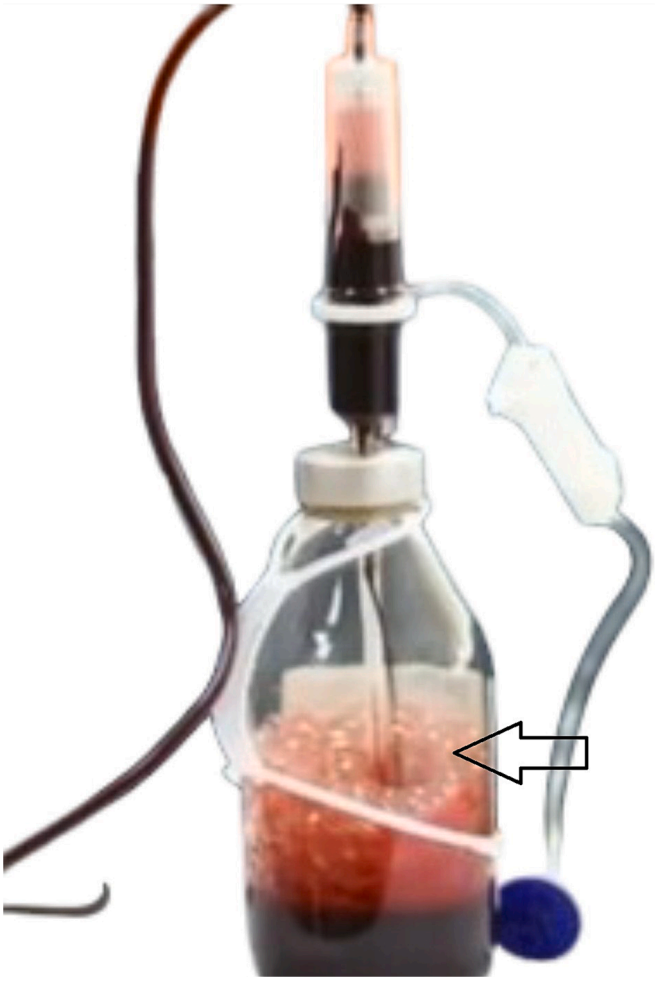

Fig. 1. Foam due to haemolysis in a non standardized oxygen-ozone therapy approach using glass bottle.

system. This may increase the risk of thrombotic events such as deep vein thrombosis (DVT), pulmonary embolism (PE), stroke, and myocardial infarction [7,8].

SIOOT recommends to fully avoid oxygen-ozone approaches capable to reach a haemolysis ≥0.1–0.5 %, to use standardized and certified phthalate-free plastic bags suited for blood transfusion, to avoid glass bottles and narrow needles for oxygen-ozone major autohaemotherapy.

Haemolysis can be a real concern for the positive outcome of oxygen-ozone therapy and for patients' safety.

CRediT authorship contribution statement

Marianno Franzini: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Project administration. **Luigi Valdenassi:** Conceptualization, Data curation, Investigation, Software, Supervision, Validation, Writing – review & editing. **Umberto Tirelli:** Supervision, Validation, Visualization. **Francesco Vaiano:** Data curation, Investigation, Methodology, Software. **Salvatore Chirumbolo:** Conceptualization, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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