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ISTH SSC COMMUNICATIONS

Monitoring and reporting the composition of plasma and serum to improve biobanks and comparability of extracellular vesicle research: communication from the ISTH SSC Subcommittee on Vascular Biology

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Abstract

Transparent reporting is key to improving the reproducibility of scientific research. In 2023, the International Society for Extracellular Vesicles updated the "*Minimal information for studies of extracellular vesicles*" (MISEV) reporting guidelines and published new recommendations for blood extracellular vesicle (EV) research entitled "*MIBlood-EV: Minimal information to enhance the quality and reproducibility of blood extracellular vesicle research.*" The MIBlood-EV recommendations are part of MISEV 2023 and promote reporting not only the protocols used for blood collection and handling but also the composition of the prepared samples that are used to measure EVs. Plasma and serum are commonly used starting materials for EV research; reporting their composition can help to improve reproducibility, comparison of measurement results, and support evidence-based guideline development. We conducted an online survey among the International Society on Thrombosis and Haemostasis (ISTH) EV researchers. Of the 20 respondents, 95% were familiar with MISEV, but 35% were unaware of the 2023

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update, and only 65% applied these guidelines to their reports. With regard to MIBlood-EV, 40% were unaware of this reporting tool, and 20% did not follow its recommendations. This is surprising because most respondents agree that preanalytical variables of blood EV research are not satisfactorily described (75%), confirm that having a standardized reporting tool is beneficial for blood EV research (90%), and consider MIBlood-EV applicable to other fields of ISTH research (80%). In this Scientific and Standardization Committee communication, we summarize the survey results, as well as the background and goals of MISEV and how MIBlood-EV can be useful to improve the reproducibility of blood research within the ISTH community.

KEYWORDS
biomarkers, blood, extracellular vesicles, MiBlood-EV, MISEV

1 | INTRODUCTION

Extracellular vesicles (EVs) are an umbrella term for all types of cell-derived vesicles [1], including "exosomes" and "ectosomes" (the latter commonly called microvesicles or microparticles). EVs are present in body fluids such as blood and urine. The biochemical composition and functions of EVs are increasingly studied, and blood is the most studied body fluid for EV research [2].

Within the International Society on Thrombosis and Haemostasis (ISTH) community, EVs have been studied for decades, particularly for their ability to initiate coagulation [3]. For example, EVs in plasma from patients with cancer and infectious diseases can expose tissue factor [4,5]. The Scientific and Standardization Committee on Vascular Biology (SSC on VB) has been actively involved in the standardization of plasma collection for EV research [6] and in the standardization of EV concentration measurements [6,7], the latter involving a fruitful and ongoing collaboration with EV experts from the International Society on Advancement of Cytometry and the International Society for EVs (ISEV). Together, they work on promoting transparent reporting, instrument calibration, interlaboratory comparison studies, and education (http://www.evflowcytometry.org/) [8–10]. Recently, the SSC on VB compared antigenic and activity assays that measure the EV-associated tissue factor antigen and activity [11].

2 | PLASMA AND SERUM COMPLEXITY HAMPERS ISOLATION AND DETECTION OF EVS

Despite the substantial progress that has been made with regard to the detection and isolation of EVs, there are, to date, no methods available that can exclusively isolate or comprehensively detect all EVs that are present in body fluids, such as plasma and serum [9]. The main challenge is that EVs themselves are highly variable in size and do not expose a "pan-marker" that can be used to identify all EVs [12]. Especially in plasma and serum, the presence of platelets and lipoproteins hampers the isolation and detection of EVs alone.

As research with freshly collected blood is not always possible, biobanks are a valuable provider of stored plasma and serum for research. While sample processing methods are usually well documented, quality control of prepared specimens is not routinely performed and reported. The relationship between sample composition and processing methods is complex since the protocols to collect blood and prepare plasma and serum vary between biobanks and laboratories [13], and the composition of plasma can differ even between laboratories using the same protocol [14].

3 | TOWARD PLASMA AND SERUM SAMPLES OF KNOWN COMPOSITION

There seem to be 2 solutions to overcome the problem of the unknown and unreported sample composition. The first solution is to standardize sample preparation. This solution is neither easy nor straightforward because there are nearly 40 variables in blood collection and handling that may affect the presence and function of blood EVs (Figure 1) [16]. Furthermore, even when laboratories use the same sample preparation protocol, the sample composition can still differ between the laboratories [14]. Thus, reporting the sample preparation protocol does not necessarily provide insight into the sample composition.

There is also another solution. In 2019, a task force of the ISEV Rigor and Standardization Subcommittee working on blood EVs came up with the idea to measure and report the composition of prepared plasma and serum samples [16]. Recently, these recommendations were incorporated into "*MIBlood-EV: Minimal information to enhance the quality and reproducibility of blood extracellular vesicle research*" [15], which is also part of the new minimal information for studies of EVs (MISEV) 2023 [1]. With MIBlood-EV, the recommended reporting guidelines specific to blood samples are outlined.

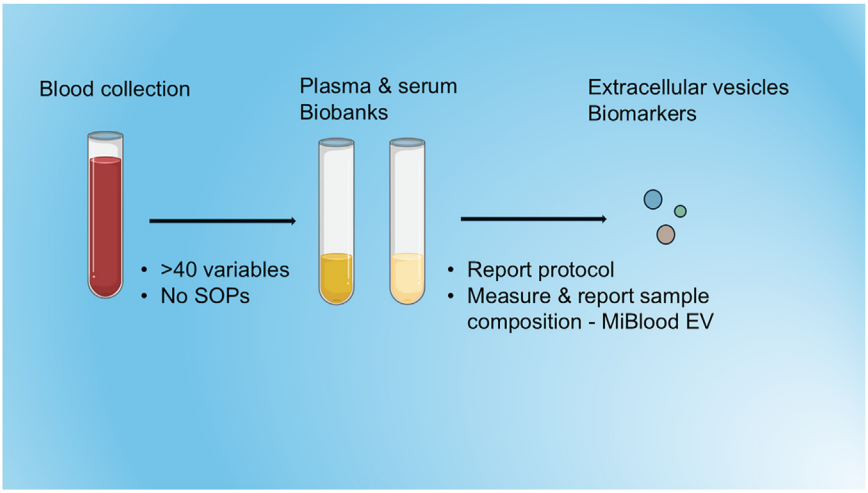

FIGURE 1 Measure and report sample composition (MIBlood-EV). In 2023, a manuscript entitled "*MIBlood-EV: Minimal information to enhance the quality and reproducibility of blood extracellular vesicle research*" [15] was published by the Blood Task Force on Extracellular Vesicles (EVs) by the Rigor and Standardization Subcommittee of the International Society for EVs (https://www.isev.org/taskforces). MiBlood-EV advocates reporting not only the protocol used for blood collection and handling but also measuring and reporting the composition of the prepared plasma and serum samples. The goals of MiBlood-EV are (i) to provide insight into the sample composition, (ii) to develop sample inclusion and exclusion criteria, (iii) to improve reproducibility without forcing researchers to change local protocols or equipment, (iv) to develop evidence-based collection and handling protocols, and (v) to improve the understanding of results between studies. Although developed for EVs, MiBlood-EV may also be useful for other biomarker studies and biorepositories. SOP, standard operating procedure.

4 | WHAT ARE MISEV AND MIBLOOD-EV?

MISEV 2023 is a consensus paper that was written by the ISEV community, has more than 1000 authors, and is a follow-up on earlier editions published in 2014 and 2018 [1,17,18]. In brief, MISEV 2023 provides an introduction to EV research and nomenclature, recommendations to improve reproducibility, information to assist reviewers and editors, examples of EV separation and detection methods, a framework to support EV research and its applications, and information to support translational and clinical research.

MIBlood-EV recommends researchers and biobanks to report the composition of the prepared plasma and serum samples (Figure 2), in this particular case with emphasis on EVs, but in principle applicable to any type of blood-related studies. The recommendations of MiBlood-EV have been incorporated into MISEV 2023, and the MiBlood-EV reporting tool is available at https://www.isev.org/assets/Rigor/TaskForces/Blood/MIBlood-EV%20v1.1.pdf.

Here, we aim to explain the background of MIBlood-EV with an example. From the perspective of EV researchers, plasma and serum are complex biofluids that contain not only EVs but also soluble proteins and non-EV particles, ie, platelets and lipoproteins. Plasma EVs are commonly studied as carriers of microRNA (miRNA), but soluble proteins, platelets, and lipoproteins are also known to carry miRNAs. Consequently, robust separation of EVs from soluble proteins and non-EV particles is essential to accurately study plasma EV-associated miRNAs. While soluble proteins can be relatively easily separated from EVs, the non-EV particles overlap in size and density with EVs, making separation highly challenging. Thus, when measuring plasma EV-associated miRNAs, the presence of confounders such as platelets and lipoproteins should be considered.

At present, the results from studies on plasma EV-associated miRNAs are incomparable, likely due to the presence of unknown amounts of platelets and lipoproteins. Moreover, because laboratories and biobanks use different centrifugation protocols to prepare their plasma and serum, the concentrations of miRNA-carrying confounders, such as platelets, can differ between laboratories, biobanks, and studies.

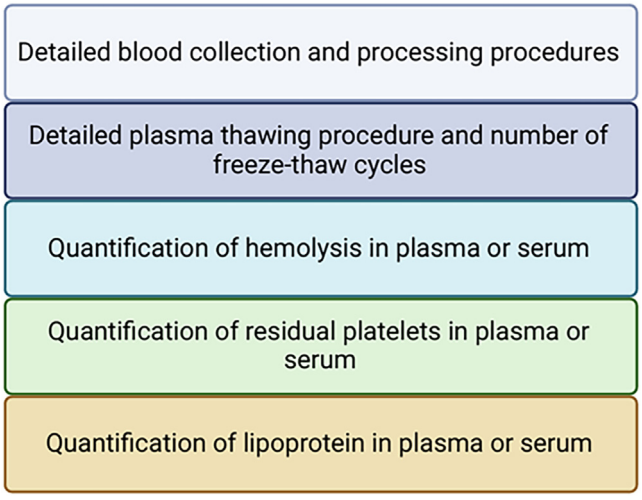

FIGURE 2 Components of MiBlood-EV.

Thus, MIBlood-EV promotes measuring and reporting the sample composition. This information provides insight into the quality and consistency of the collected samples and may be helpful in comparing and/or understanding differences in results between studies. Obviously, whether a particular plasma or serum compound is a confounder or not depends on the research question and the analyte of interest.

5 | WHAT ARE THE ADVANTAGES OF MIBLOOD-EV?

MIBlood-EV is not intended to assess study or methodological rigor. In addition, it is not intended to be used as a tool to determine the inclusion or exclusion of clinical samples based on their quality. Clinical samples are often valuable, and by measuring and reporting sample composition, the obtained results can be even better evaluated. What are, then, the advantages of measuring and reporting the composition of plasma and serum samples?

First, measuring the sample composition provides direct insight into the presence of potential confounders. This is also useful for internal use, for example, to check the variability between operators or the performance of equipment. For example, we (ie, the Amsterdam Vesicle Centre) observed differences in the plasma concentration of platelets between operators. It is notable that operators used the same protocol and centrifuge. Platelets are a source of miRNAs, and their presence affects measurements of "EV" miRNA [19]. Therefore, the platelet concentration should be as low and consistent as possible. This problem was overcome by using a Lego brick, which is about 1 cm high. All operators now use this brick to mark their tubes 1 cm above the buffy coat or cell pellet as a visual guide for pipetting [12]. A second example is that plasma prepared in one particular laboratory had a 1000-fold higher platelet concentration compared with other laboratories using the same protocol because their centrifuge lacked a switch to disable the brake [14]. Taken together, measuring the concentration of the remaining platelets in plasma is a useful checkpoint to optimize local procedures and check equipment.

Second, by measuring and reporting the composition, criteria can be developed for sample inclusion or exclusion. The development of such criteria, which may depend on downstream applications, such as for studying miRNA, may also be helpful in case samples from different studies, biobanks, and laboratories that need to be measured together.

Third, by introducing relevant checks, there is no need to change local procedures and infrastructure. By monitoring the sample composition, the focus shifts from standardization to reproducibility, which is, in the end, what matters most.

Fourth, although this manuscript is written from our perspective of working with blood-derived EVs, we recognize that a broader interest in the composition and consistency of plasma and serum samples will also be beneficial to other fields of research. For example, with regard to the isolation of specific biomarkers, such as cell-free DNA or tumor-educated platelets, currently used centrifugation protocols enrich the biomarkers of interest rather than purify those, and knowing and quantifying the presence of relevant confounders may help to better understand and interpret the results [20]. This effort is also underway for the measurement of neutrophil extracellular traps through the SSC on VB.

Finally, we believe that transparently reporting the composition of plasma and serum samples will help with understanding the often incomparability of results between (often) single-center studies. Also, we are in the process of setting up an online biorepository, with the goal of developing future evidence-based guidelines when sufficient data becomes available. In principle, such an online biorepository could be integrated into an already existing online database, such as EV Track (www.evtrack.org).

6 | RESULTS FROM A SURVEY AMONG THE ISTH MEMBERSHIP

In October 2024, the SSC on VB organized a REDCap survey circulated to ISTH members to ask for their input and viewpoint on the current status of EV research and reporting, whether they are aware of MISEV and MIBlood-EV, and whether they think the MIBlood-EV approach may be useful to ISTH-relevant topics other than EVs.

The main outcome of this survey, which was completed by 20 experts in the field of EV research within the ISTH community, is shown in Figure 3. Overall, 95% of the respondents were aware of MISEV guidelines, 35% were unaware of its recent update, and only 65% used these guidelines within their own publications.

With regard to MIBlood-EV, 40% were unaware of this reporting tool, and 20% did not follow its recommendations. These results are surprising because 75% of the respondents think that preanalytical variables of blood EV research are not satisfactorily described, and 90% agree that having a standardized reporting tool would be beneficial for blood EV research. When asked, "*How do you think MiBlood-EV can be most helpful for standardizing extracellular vesicle research?*" 80% of the respondents answered increasing transparency in reporting for publication, followed by improving the reproducibility of EV research (75%), standardizing preanalytical variables (70.0%), providing information to collaborators or biobanks for sample collection (70.0%), reporting quality of blood, plasma, and serum samples (65.0%), providing information to collaborators or biobanks for sample storage (60.0%), and providing information to collaborators or biobanks for sample processing/analysis (55.0%). Finally, 80% considered MIBlood-EV also applicable to ISTH topics beyond EVs. Supplementary Figures S1–S9 provide a detailed overview of the full survey results.

Overall, there is strong support for a standardized reporting tool, such as MIBlood-EVs, among members of the ISTH community working on EVs. This will improve not only the transparency of reporting and reproducibility of blood EV research but also the standardization of preanalytic and relevant information for biobank samples.

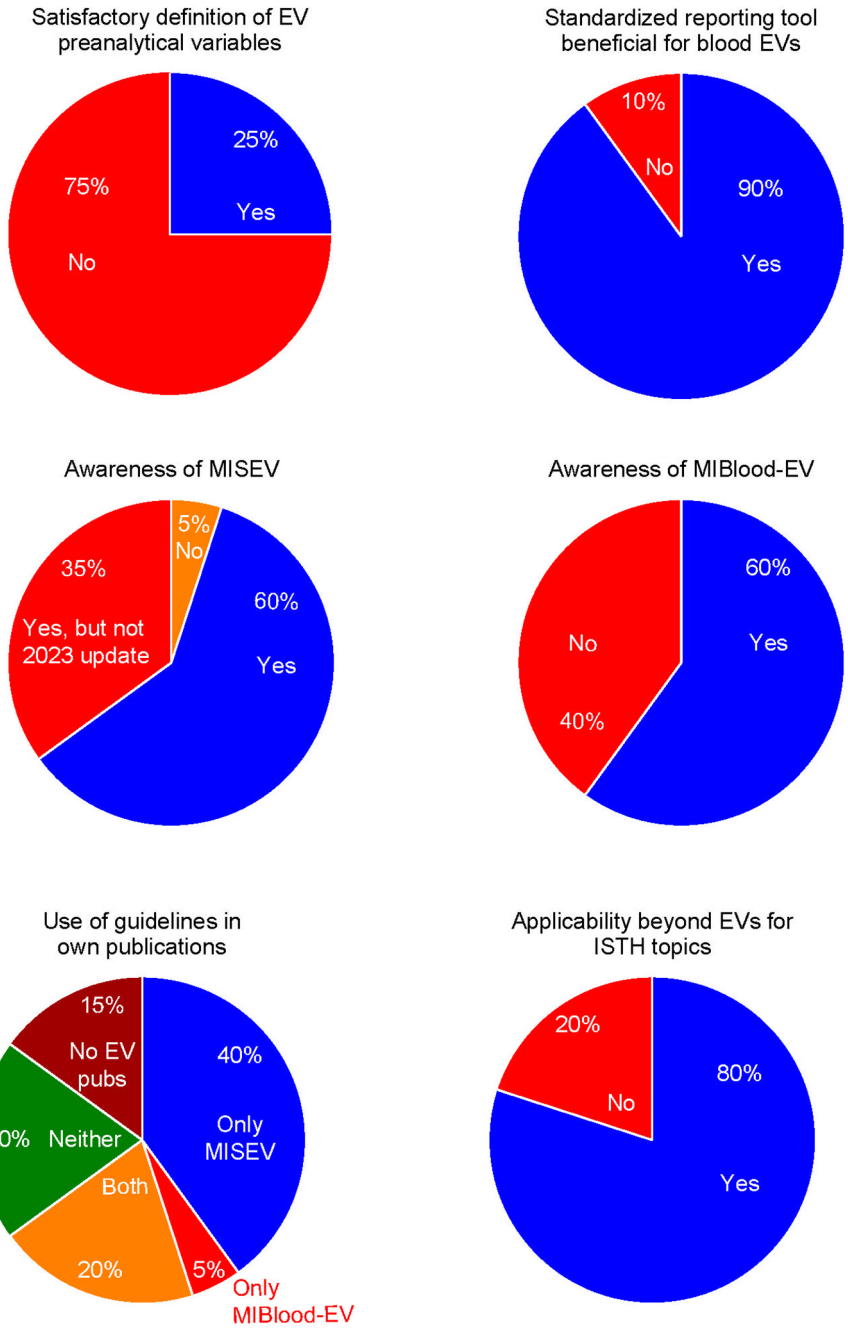

FIGURE 3 Results from a survey circulated to the International Society on Thrombosis and Haemostasis (ISTH) members working with extracellular vesicles (EVs) about reporting awareness. Twenty individuals with expertise in EV research responded to a survey circulated by the Scientific and Standardization Committee on Vascular Biology after an open call to the ISTH community. Respondents were asked to choose 1 response related to their views on the current state of EV research and reporting, their awareness of the minimal information for studies of EVs (MISEV) and MIBlood-EV and their use in their own publications, as well as if this approach could be applicable to other ISTH-relevant topics beyond EVs. For an overview of all Survey results, please see the Supplementary Material.

7 | SUMMARY

Taken together, we hope that measuring and reporting the composition of plasma and serum samples becomes an intrinsic element of the "Materials and Methods" section of high-quality scientific journals, which will be helpful in improving transparency and reproducibility.

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AUTHOR CONTRIBUTIONS

Draft was prepared by R.N. Y.H. and K.M. corrected the draft and prepared the figures. K.M. developed and analyzed an online survey for the ISTH membership with help from M.L. M.L., D.G., and F.L. helped to prepare the final version of the manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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