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Review

Improving Traditional Post Mortem Healthcare—The Cross-Sectional Use of Blood-Based Biomarkers

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Abstract: Many tools of clinical medicine, such as clinical chemistry and diagnostic imaging, are prioritized for clinical diagnosis over post mortem diagnosis. Indeed, it is reasonable that the assessment of a patient's functional status should take priority over the post mortem, cross-sectional use of diagnostic tests and laboratory equipment. In addition, these tools are sometimes expensive, and their use does not always have a reasonable cost–benefit ratio. However, some post mortem observations, such as inflammation, pulmonary edema, or infiltration and cerebral swelling, cannot be explained without using immunohistochemical markers for post mortem diagnosis. Introducing blood-based biomarkers into post mortem care could significantly reduce the rates of inconclusive post mortems and discrepancies in autopsy findings and clinical diagnoses. This is particularly relevant in relation to vascular pathology, considering the significant burden that vascular diseases represent for overall mortality. Expanding traditional autopsies with blood-based (circulating) biomarkers to avoid invasive post mortem examination would have cultural, religious, and potentially economic advantages. All of the target molecules were discussed in the context of the processes they up-regulate or down-regulate, which turned out to be the final cause of death. Ultimately, it is evident that further studies are needed to provide concrete validation for using a combination of markers for each case to reach a post mortem diagnosis with or without clinical records.

Keywords: blood-based biomarkers; clotting; inflammation; post mortem

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1. Introduction

Biomarkers found in bodily fluids may represent the active disease process or the patient's reaction to that disease [1]. Moreover, they can act as an alternative measure of outcomes to assess the efficacy of therapy. According to common wisdom, a biomarker is a protein, enzyme, or cytokine with discriminatory value in clinical care [2,3]. A variety of molecules have been evaluated, and although post mortem biomarkers and a multimarker strategy are best investigated in the light of sudden cardiac death and agonal cardiac function [4,5], their significant potential in relation to peripheral vasculature is yet to be addressed [1,6]. All biomarkers must meet certain criteria to constitute a surrogate endpoint, or to be able to predict a clinically relevant endpoint, such as the loss of vision or a decrease in quality of life. In addition, the effect of a proposed treatment on the surrogate must capture the effect of the treatment on the clinically relevant endpoint [7,8].

This information should be considered in the context of the fact that autopsies face a number of challenges; for example, the lack of regulation for governmental funding for hospital-based autopsies, or hospitals rejecting autopsies requested by families [9]. In any case, autopsy numbers have fallen significantly worldwide (Figure 1) [10–14], and the accessibility of post mortem healthcare is uneven [15]. It is necessary to improve these statistics and also to address the major problem of discrepancies between clinical diagnosis and initial autopsy findings regarding the panel of clinical biomarkers. This discrepancy ranges from 7.2% in a 1993 study by Stambouly et al. to 64% in Mitrovic et al., 2019 [16,17].

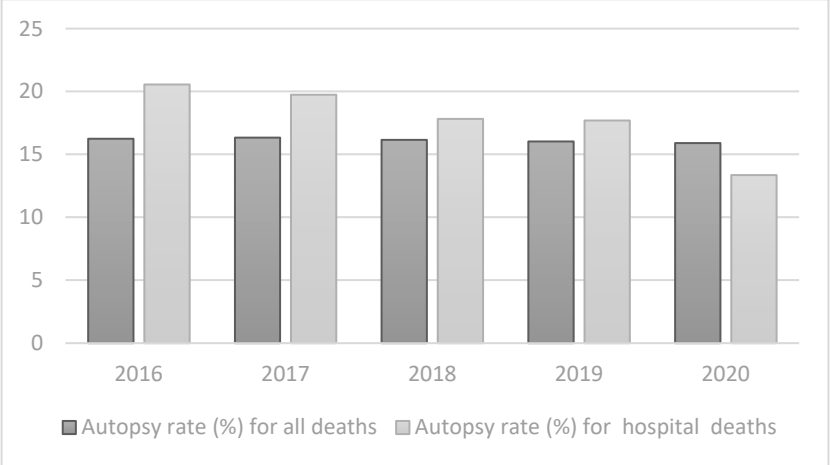

Figure 1. Five-year autopsy rate for all-death mortality worldwide; 2016–2020, slight decline in the overall autopsy rate, and a more evident decrease in the hospital death/autopsy rate (for more than 4% for the year 2020 compared to 2019).

Compelling grounds for this review was improving the standard of post mortem healthcare. The use of biomarkers as a replacement or addition to traditional autopsy (TA) should help in dodging the huge number of inconclusive or discrepant autopsies. Duly, the data known at present on blood biomolecules, which make it possible to determine the cause of death, will be reviewed, and minimally invasive approaches to postmortems will be tackled. Thus, invasive procedures that require the full opening of the body when performing an autopsy may be avoided [18–20].

2. Traditional Post Mortem Healthcare

Despite its discrepancies with clinical records, autopsy remains the gold standard as the ultimate diagnostic procedure [21,22]. Although these discrepancies have decreased significantly over time, in 2010 their rate remained high [23]; in the "post-COVID" era, the rate has reached an unprecedented 42% [24]. This renders between one in two and one in three autopsies superfluous.

Our knowledge about normal circulation stems entirely from thorough post mortem dissection [25]. More than 40 years ago, in a series of 500 clinical autopsies, vascular disorders were found to account for 25.2% of anatomopathological diagnoses [26]. These figures were more or less the same in osteoarthritis/rheumatoid arthritis research from 2015 [27]. Data from the Eurostat indicate the same phenomenon: diseases of the circulatory system are the main cause of death in the EU and were responsible for almost 37% of all deaths in 2017 [28,29]. A biomarker may be a recording taken from an individual, an imaging test, or a biosample.

Earle et al. recently presented data on the cause of death in patients with a risk of pulmonary embolism (PE), and their figures are instructive [30]; PE was excluded using clinical decision-making rules in combination with a D-dimer assay (the D-dimer is so named because two D fragments of the fibrin protein are joined by a cross-link) [31]. A lack of circulating oxygen, altered enzymatic reactions, cellular degradation, and the cessation of the anabolic production of metabolites all caused extensive biochemical changes in all body tissues post mortem [32]. Aside from its implications for PE, the value of D-dimer as a biomarker was revealed during the coronavirus disease 2019 (COVID-19) pandemic, when it was used to assess patients for disease severity and mortality in a case–control study [33].

Etymologically, the term "biomarker" comes from the Greek form βιο-, from βίος, meaning life, and the Old English word meaning a mark [34,35]. Bearing in mind this Greek root, using the word 'life' in the context of a post mortem may seem slightly incongruous.

This was the case until recently, when the COVID-19 pandemic brought about a radical shift in routine post mortem practice [36].

3. Options for Traditional Autopsy

Traditional autopsy may be criticized in the media, but it is an important tool for both criminal investigations and healthcare quality control. For this reason, minimally invasive alternatives to traditional autopsies are continuously emerging. Imaging and "verbal autopsy" (VA) were shown in a large series to be promising techniques compared with a full autopsy [37–40] (Figure 2). Various objective factors influence the autopsy rate, though it is less likely to be requested for deaths in the emergency department or on general surgery wards, and it is most likely to be requested for fetal, medicine-related, cardiothoracic surgery-related, and pediatric deaths [41]. Nevertheless, most countries globally do not report high autopsy rates (less than 70% of all-cause mortality) [42].

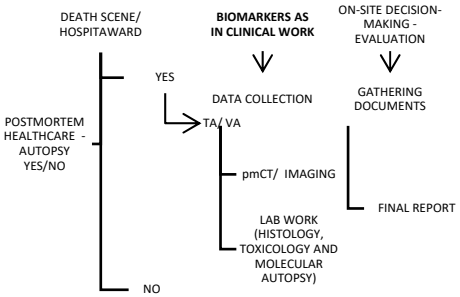

Figure 2. Schematic of a provisional post mortem protocol created by the author, with biomarkers included; TA—traditional autopsy; VA—verbal autopsy.

While the cost of electronic data systems and the long wait between data collection and analysis appear to be the main disadvantages of verbal autopsies, post mortem imaging is hampered by a lack of direct visualization of the soft tissue, as well as postmortem artifacts that obscure the natural causes of death and can be misinterpreted as antemortem pathologies [40,43]. However, VA has been preferred recently in the COVID-19-related pandemic context, with a satisfying effect [44]. Additionally, it is not invasive procedure, so it does not require the opening of the body when performing an autopsy [18–20]. Trained interviewers can use a questionnaire to interview the caregivers of the deceased. Due to its non-contact nature, the World Health Organization's (WHO's) declaration of COVID-19 as a pandemic constituted an opportunity to make use of the VA technique [45,46].

For deaths that occur outside the health system, health information and a description of the events preceding death are included in the VA. It was first used in a public health project concerning the relationships between nutrition, infection, and child development in India [47]. Nowadays, this method has been improved and augmented so that it yields suitably complete death certificates and ultimately estimates cause-specific mortality. Specifically, VA means the collection of anamnestic data through an in-person interview with a close relative or caregiver of a deceased. The interview takes place within a short time of death; these data include symptoms, signs, and circumstances prior to death [48]. In settings where most deaths are otherwise undocumented, which typically means in low- and middle-income countries, VA attempts to establish causes of death, allowing scientists to analyze disease patterns and direct public health policy decisions. The body of relevant literature reports that the specificity of the VA is commonly found to be higher than sensitivity [49]. Additionally, the negative predictive value (NPV) was higher than the positive predictive one [50]. When assessing the cost of VA in rural India, the total cost per death was USD 16.66 [51]. The annual cost for the whole population included in the study in year 1 was USD 24,943, inclusive of training. The average annual cost to run the system each year was USD 18104, and the cost per death was USD 12 for the next 3 years. Costs were reduced by using single-physician reviews and shortened re-training sessions.

In agreement with contemporary attainments, even conducting an autopsy can be transferred to a computerized environment, and digital tools can be employed. Accordingly, another accessible and recently developed modality of postmortem healthcare is a radiographic examination of the body after death—postmortem radiology. As much as they provide a strong complementary tool to the TA, imaging techniques used in everyday clinical work are applied to post mortem processing [43]. In cases where forensic radiology plays a primary ancillary role in the post mortem, this directs forensic pathologists to specific screening tests [52,53]. Various imaging techniques can be considered relatively reliable when the patient in the medicolegal setting needs to be assessed. For instance, post mortem cardiac magnetic resonance imaging offers a better insight into the cardiovascular diseases responsible for sudden cardiac death (SCD) [54]. Forensic pathologists can benefit from these tools, even when an autopsy, fluid analysis, and DNA sampling are required [55,56]. In most countries, at minimum post mortem Computed Tomography (PMCT) imaging is regarded as accessible, reproducible, reliable, and easy to implement.

The explicit potential economic benefits of the PMCT (magnetic resonance imaging—MRI) have not been assessed recently [38,57]; despite its numerous advantages, this method still exhibits the problem of a significant rate of diagnostic discrepancies [58,59]. Nevertheless, PMCT has 79% sensitivity and 92.1% specificity for the detection of the source of bleeding [60]. In another study, where MRI and ultrasound (US) were used as imaging modalities, no significant difference in the rates of agreement was reported [61]. A study that assessed the diagnostic accuracy of post mortem imaging claimed that the mean cost of TA was 70% more expensive; as such, having post mortem imaging available would leave the institution performing the autopsy with more funds [62].

4. Post Mortem Biomarkers

Biomarkers provide plenty of information for enhancing all aspects of vascular homeostasis through vascular beds [1]. Biomarkers are characteristic indicators of disease, a disease state, or disease progression. They were at first described as a "measurable and quantifiable biological parameter that could serve as an index for health assessment" and were ultimately defined as "a characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [63,64].

The post mortem period involves events such as autolysis or decay, and biomarkers found in bodily fluids may represent the progression of the active disease or a reaction to the disease. Therefore, the value of post mortem biomarkers should be evaluated with this in mind, even if their efficacy is clinically confirmed [65]. This compounds the value of clinical post mortem studies as not only a method of control but also a means of improving teaching methods in hospitals [13]. The augmentation of post mortems with blood-based (circulating) biomarkers, in order to avoid invasive autopsies, would have cultural, religious, and potentially economic benefits [38,57,66].

In fact, no contemporary studies compare the costs of the various post mortem optional modalities.

5. Biomarkers of Vascular Quiescence

Endothelial quiescence and normality are important for disease resistance. Circulating blood-based biomarkers are simply signs of organ-specific signaling pathways [67]. The vascular system has a resting layer of endothelial cells (EC) that does not divide. Moreover, this layer of long-lived cells of the mesodermal lineage, which line the inside of all blood vessels, forms a single layer of organotypically differentiated cells [68]. This is known as vascular quiescence, and little is known about how the body achieves and maintains it.

5.1. Circulating Markers of the Extracellular Matrix: Biomarkers Related to the Vascular Wall

Collagen fragmentation is typically found in abdominal aortic aneurysm (AAA) biopsies as an indicator of new types I and III collagen synthesis [69]. AAA is interesting in the

context of post mortems since it bears the risk of a rupture or a dissection—life-threatening conditions with high mortality rates [70,71]. This mortality is about 25% at 6 h and rises to 50% by 24 h; this can be compared to the rates of 40–70% in cases of sepsis [72,73]. Therefore, the search for highly sensitive and specific biomarkers for AAA should be equally focused.

Both the carboxy-terminal and amino-terminal ends of the precursor molecule are released during collagen synthesis, and fragments represent candidate biomarkers. A larger study and confirmation of clinical validity in a larger cohort is needed to link these molecules to AAA. In that regard, another candidate biomarker that has been suggested is tenascin-X, due to its involvement in Ehlers–Danlos syndrome. AAA patients showed elevated serum levels compared to controls [74,75]. Considering that serum elastin peptide (SEP) is a degradation product of elastin, its role as a biomarker has been shifted from sepsis to the extracellular matrix in vascular quiescence [76,77].

Furthermore, the examination of the wall of aortic aneurysms has demonstrated medial arterial destruction, the accumulation of inflammatory cells, the fragmentation of elastin, increased concentrations of proteolytic cytokines, and an in situ thrombus [78]. As such, some additional enzymes, proteins, and cytokines have been explored in relation to this finding. This approach has most often been limited by the fact that all these features represent the end-stage of AAA development, and may not be indicative of the factors that initiate AAA development or stimulate AAA growth.

The fragmentation of the extracellular matrix implies the involvement of elastases and matrix metalloproteinases (MMPs) in the pathophysiology of AAAs. As AAAs are a setting for the abundant expression of the MMP-9, it is considered to play a pivotal role in their formation. Therefore, this enzyme was explored as a possible biomarker for the presence of AAA in case–control studies. Patients with AAA demonstrated elevated concentrations of circulating MMP-9 [79]. The possible use of elastases as serum biomarkers of extracellular matrix remodeling is the basis of some studies involving alpha-1 antitrypsin or p-elastase [80–82]. However, the short half-life of active MMP-9 implies that any active MMP-9 in the serum may have a more immediate origin, so this information could be relevant to clinical forensic scientists [83].

Higher MMP-9 levels are associated with plaque vulnerability in carotid artery atherosclerosis [84]. This is the result of an interaction between modified lipids, the extracellular matrix, macrophages, and activated vascular smooth muscle cells (VSMCs). Inflammation, lipid accumulation, apoptosis, thrombosis, angiogenesis, and proteolysis all take part in the evolution of atherosclerotic lesions, as these processes are linked to the morphological characteristics of an unstable plaque. Therefore, the search for a biomarker has focused on these processes [85]. The interplay of vascular wall remodeling and carotid pathology was first hinted at by Makita et al., who drew a link between CRP levels with the carotid intima–media complex thickness and plaque formation [86]. Today, there is a link between obesity in children and adolescents and MMP-9 [82,87]. On the other hand, decreases in MMP-3 and MMP-9 have been reported after successful endovascular repair [88,89]. However, these data have highly limited post mortem significance.

Biomarkers are actively sought out for diseases that damage society in developed countries (e.g., dementia, renal and cardiovascular disease, and most malignancies). Unfortunately, all the studies on this topic have involved small numbers of patients and similar numbers of control subjects [90,91]. Finding appropriately matched controls is a real challenge that decreases the odds of clinical validation. This is most often the case when aortic wall tissue is used in proteomics; it is difficult to obtain a normal-aged aorta to use as a control. Even if such tissue is obtained, the method and timing of its harvest and preservation will modify its protein expression.

5.2. Proteins Associated with Vascular Lumen: Inflammation and Thrombosis Biomarkers

Whether as the final product or an outgrowth of the signaling pathway of degradation, markers of inflammation in vascular disease include cell adhesion molecules, cytokines, pro-atherogenic enzymes, and CRP [82,92]. Biomarkers used to identify thrombosis are

unlikely to translate into a universal clinical tool; conversely, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and procalcitonin (PCT) are often used [93]. Moreover, hyperhomocysteinemia has been identified as an indicator of oxidant stress and a significant cardiovascular risk factor [94,95], although this association is weak.

The principal markers that have been evaluated are fibrinogen, D-dimer, homocysteine, and CRP, the elevation of which is intimately linked to other inflammatory cytokines, including interleukins (ILs; e.g., IL-6) and macrophage activation [96,97]. Assessing protein complexes embedded in the coagulation cascade and CRP levels, which are elevated in large aneurysms, covers both processes [98]. CRP levels decrease quickly, with a half-life of about 19 h [99].

Out of all the acute-phase proteins, CRP is the most commonly investigated biomarker in vascular pathology. Its specific role is to activate the complement cascade in cell death [100], and it is inextricably linked to other inflammatory cytokines [97]. One such cytokine is IL-6, which was confirmed to be a product of AAA [101]. It is even present in uncomplicated thoracic aortic aneurysms, since the C-reactive protein/interleukin-6 ratio may be a marker of the size of the aneurysms [102]. Additionally, plasma IL-6 has been correlated with aortic diameter in patients without AAA [6].

Combined with CRP, PCT was tested as a biomarker for sepsis [103]. In terms of the diagnostic accuracy of using CRP as a marker for sepsis, the overall area under the summary receiver operator characteristic (SROC) curve was 0.73 (95% confidence interval (CI), 0.69–0.77), with a sensitivity and specificity of 0.80 (95% CI, 0.63–0.90) and 0.61 (95% CI, 0.50–0.72), respectively; the DOR was 6.89 (95% CI, 3.86–12.31). In terms of the diagnostic accuracy of using PCT for sepsis, the overall area under the SROC curve was 0.85 (95% CI, 0.82–0.88), with a sensitivity and specificity of 0.80 (95% CI, 0.69–0.87) and 0.77 (95% CI, 0.60–0.88), respectively, and the DOR was 12.50 (95% CI, 3.65–42.80) [104,105].

The molecular basis of blood coagulation first attracted attention in the search for blood-based biomarkers due to a plasma fibrinogen concentration that was positively correlated with the AAA diameter [106]. Nonetheless, its elevated plasma concentrations are induced by smoking, so the association can only be linked to the "black box" of smoking [107]. Due to various functional interactions, fibrinogen plays a crucial role in hemostasis. Specifically, it is a substrate for three major enzymes: thrombin, plasmin, and factor XIIIa [106].

As the clotting slows down, the clot breaks down and, together with the fibrin net, it ultimately dissolves. With this dissolution, fragments of protein are released into the bloodstream. One such specific fragment, which is formed only upon the degradation of cross-linked fibrin, is D-dimer [108]. Plasma concentrations of D-dimer show fibrin turnover in the circulation and are ultimately related to subsequent mortality from any cause [109]. Most importantly, the D-dimer level is a validated assay that is routinely used in general clinical practice to exclude a diagnosis of deep vein thrombosis (DVT) [110]. The current serum levels of D-dimer are directly proportional to recent fibrinolytic activity, as the half-life of D-dimer is four to six hours, and its levels stay elevated for about seven days [111]. This information could have great forensic value in the context of defining the time of death or establishing a chronological timeline [112]. Hence, the measurement of post mortem D-dimer may lead to a certain practical improvement in current post mortem healthcare.

The currently available D-dimer assays are not standardized and it is unclear whether these differences have an impact. On the other hand, these tests are rapid, simple, and inexpensive [113]. Therefore, to explore the differences between D-dimer assays and their impact on the diagnostic outcome, a prospective multicenter cohort outcome study evaluating 3462 patients with suspected PE (the YEARS study) was conducted. Four different D-dimer assays were used, and the median D-dimer concentrations differed significantly between the assays. The sensitivity, specificity, positive predictive value (PPV), and NPV for the detection of PE of all four assays were determined, using a cutoff level of 1000 ng/mL [31]. In post mortem blood, an immunochromatographic SERATEC PMB test was used [114].

This test targets human hemoglobin and D-dimer simultaneously, so it is used in forensic inquests for menstrual and peripheral blood spatters [115].

CRP and D-dimer are of significant interest, as they are widely used in clinical work [116]. While the role of both of these molecules as candidate biomarkers in clinical work has been explored, their use in post mortem processing is more a matter of the pathologist's discretion.

6. Vascular Cognitive Impairment: Room for Biomarkers at Post Mortem

Vascular cognitive impairment (VCI) is a term used to encompass the entire spectrum of cognitive disorders related to the mental abilities of awareness, thinking, and feeling. It is associated with a variety of cerebral vascular brain injuries. VCI symptoms can range from forgetfulness to more serious problems with attention, memory, language, and executive functions such as problem solving. Cerebrovascular disease (CeVD) and neurodegenerative forms of dementia, such as Alzheimer's disease (AD), are frequently associated comorbidities in the elderly, with similar risk factors and pathophysiological mechanisms, including neuroinflammation [117].

As an inflammatory marker that is upregulated in vascular diseases, as well as in AD, protein secreted to plasma (i.e., osteopontin (OPN)) has been tested as a biomarker of AD and VCI [118,119]. OPN's involvement in lipid metabolism likely explains its role in conditions that fall under the spectrum of VCI. Moreover, among its numerous functions, OPN has emerged as an important potential biomarker for diagnosing and monitoring the treatment of cancer (including melanoma, breast, lung, gastric, and ovarian cancers) and other conditions [120,121].

Potentially relevant to practitioners is the fact that, by neutralizing OPN with various therapeutic antibody modalities, it is possible to conclude that the half-life of OPN differs depending on the antibody ligand interactions, pH, or "sweeper" used. The calculated half-lives for these four proteins range from 5 to 15 h [122].

7. Applying Clinical Biomarkers in a Post Mortem Setting

Applying clinical biomarkers in a post mortem setting does not violate the medicolegal requirements for death investigations. Nevertheless, instead of limiting the contents of the death investigation toolbox, biomarkers could be used to decrease the rate of clinical–autopsy discrepancies and to reduce post mortem healthcare inequalities [12,123,124].

At the time of this review, only a few countries had published data for both the autopsy rate and gross national product (GNP), so the correlation between the number of autopsies and GNP was weak and negative ($r^2 = -0.38$; $p = 0.004$). Subject discrepancies were minimized over time and then increased significantly in the last few years [24,125]. One recent study found that there was no significant difference in treatment time between hemorrhagic and ischemic lesions seen later at brain autopsy (unpublished data [126]).

Predominately as a consequence of the decline in rates of clinical (hospital) autopsies, overall autopsy rates have declined in recent decades in many high-income countries [127]. This negative trend has been attributed to various factors such as costs, a lack of medical education, the development of new clinical diagnostic tools, medical malpractice implications, and difficulties in obtaining permission from relatives [128]. Even if performed, autopsies tend to be negative, failing to produce findings that reveal the cause of death. On the other hand, studies show substantial discrepancies between autopsy results and pre-mortal clinical diagnoses [21,129]. This is most clearly visible in the global autopsy rates in all-cause mortality, part of the World Health Organization's (WHO's) annual statistics [10,11]. Paratz et al. reported that these rates ranged from 0.01% to 83.9%, based on the data from the few countries—less than one-third worldwide—that report autopsy rates [42]. Their statistics are mostly derived from academic journals, rather than governmental data.

Healthcare practices have come a long way in reducing mortality, but the decreasing number of TAs demonstrates the need for a feasible alternative. Nonetheless, any form of post mortem investigative tool can provide additional information or a change in diagnosis

regarding the cause of death in a great number of cases, either because of discrepancies between the clinical and autopsy diagnoses or through inconclusive autopsies. In order to maintain the viability of academic departments involved in post mortem care and to increase consent in post mortem investigations, a panel of noninvasive biomarkers is given in Table 1.

Table 1. Possible blood-based biomarkers of vascular disease.

Related Process	Biomarker	Medium	Reference Values	Half-Life
Inflammation	CRP	S	<0.3 mg/dL: normal 0.3 to 1.0 mg/dL: normal to minor elevation (can be seen in obesity, pregnancy, diabetes, common cold, gingivitis, periodontitis, sedentary lifestyle, cigarette smoking, and genetic polymorphisms) [130].	~19 h [99]
	OPT	S	122.3 ± 39.2 ng/mL	5 to 15 h [122]
		P	463.7 ng/mL–587.0 ng/mL [131].	
Related to thrombus	D-dimer	S	<2152 ng/mL [132].	4 to 6 h [111]
Matrix-degrading enzymes	MMP-9	S	436 ng/mL (range, 169–705 ng/mL) [133].	Short [83]

S—serum; P—plasma; CRP—C-reactive protein; OPT—osteopontin; MMP—matrix metalloproteinases.

8. Conclusions

Autopsies are still needed for the determination and correction of causes of death, even in "clear-cut" cases. Moreover, post mortem sample handling and analysis are challenges that need to be addressed, as they can produce variability in the findings; for this reason, validation with biomarkers is of key importance. There are some limitations to this review because no published large-scale study has considered post mortem human blood samples. The risks of bias includes the inability to verify the reported figures, heterogeneity in the reporting of clinical versus medicolegal autopsies, and the limited number of studies specifically concerning overall vascular pathology.

Considering the half-lives of all the candidate molecules discussed in this review, it is not likely that any of these molecules will see wider usage. However, each of the highlighted markers could prove useful in confirming or ruling out a cause of death in cases of witnessed deaths or in situations where TA is not an option. In conclusion, further work is required in the search for a new candidate molecule.

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