Protein biomarkers in blood reflect the interrelationships between stroke outcome, inflammation, coagulation, adhesion, senescence and cancer

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Abstract

The most important predictors for outcomes after ischemic stroke, that is, for health deterioration and death, are chronological age and stroke severity; gender, genetics and environmental factors also play a role. Of all these, only the latter can be influenced after the event, even though recurrent stroke may be prevented by antiaggregant/anticoagulant therapy, angioplasty of high-grade stenoses, and treatment of cardiovascular risk factors. Moreover, blood cell composition and protein biomarkers such as C-reactive protein or interleukins in serum/plasma are frequently considered as biomarkers of outcome, and they are connected to underlying molecular mechanisms such as inflammation, hypercoagulation, and cellular senescence. We surveyed protein biomarkers that were reported to be predictive for outcome after ischemic stroke, specifically considering biomarkers that predict long-term outcome (≥3 months) and that are measured over the first days following the event. We classified the protein biomarkers as immune☐inflammatory, coagulation-related, and adhesion-related biomarkers. Some of these biomarkers are closely related to cellular senescence and, in particular, to the inflammatory processes that can be triggered by senescent cells. Moreover, the processes that underlie inflammation, hypercoagulation and cellular senescence connect stroke to cancer, and biomarkers of cancer-associated thromboembolism, as well as of sarcopenia, overlap strongly with the biomarkers discussed here. Finally, we demonstrate that many of the outcome-predicting protein biomarkers form a close-meshed functional interaction network, suggesting that the outcome after stroke is partially determined by an interplay of the molecular processes relating to inflammation, coagulation, cell adhesion and cellular senescence.

Introduction

Stroke biomarkers, specifically predictors of outcome after ischemic stroke (stroke, for short), are an active and important area of research. The prevalence, loss of quality-of-life, reduction in life expectancy, and the limited treatment options after the event, are all responsible for its high socio-economic burden (Uivarosan *et al.*, 2020). Traditionally, chronological age and stroke severity were investigated alongside gender, genetics and environmental factors such as smoking (Donkor, 2018). For primary prevention in particular, further environmental factors such as diet or lifestyle are important (O'Donnell *et al.*, 2016). In terms of secondary prevention, antiaggregant/anticoagulant therapy, angioplasty of high-grade stenoses of cerebral arteries, and medical treatment of cardiovascular risk factors such as hyperlipidemia, diabetes and arterial hypertension are administered, in particular if risk factors such as arteriosclerotic stenosis, atrial fibrillation, hypertension or dyslipidemia are present (Hankey, 2014). Tertiary prevention, e.g. of dementia and frailty, includes cognitive and somatic rehabilitation (Draaisma *et al.*, 2020).

To tailor preventive strategies to the patient, biomarkers are essential. Optimally, these predict future disease, dysfunction or death, provide hints at underlying causal mechanisms, and predict the results of interventions. Biological age is a general predictor of (comorbid) disease and dysfunction (Fuellen *et al.*, 2020). Estimated by an epigenetic (DNA methylation) assay, it contributes to outcome prediction after stroke (Soriano-Tarraga *et al.*, 2021), and patients with a high biological age may receive more frequent care. Other predictors are based on gene expression (Sykes *et al.*, 2021) and protein abundance (Montellano *et al.*, 2021); analysis of proteins is generally established. Proteins are expected to be involved directly in the molecular processes that influence the outcome, and they are informative about molecular mechanisms and intervention success rates, and therefore we focus on those in this review. We only consider other biomarkers if these are connected to proteins, by forming biomarker signatures with them. The mechanistic role that can be ascribed to proteins allows two important steps towards a better understanding of their function as biomarkers for the natural history of the progression of disease: First, we can assign the proteins to specific molecular/cellular processes deemed important for the health deterioration observed after stroke. Here, we follow up upon the classification by Lehmann *et al.* (2021), considering immune□inflammatory, coagulation-related, and adhesion-related proteins. Second, we can generate a protein interaction network from the proteins that describes their molecular interrelationships. Here, we employ the STRING (Jensen *et al.*, 2009) database, and the resulting functional interaction network describes how the proteins, and therefore the molecular/cellular processes they participate in, are intertwined.

Data collection and tabulation: Blood biomarkers of stroke relate to immunity, inflammation, coagulation, and adhesion

For this narrative review, we surveyed the literature on protein biomarkers for human ischemic stroke outcome prediction in adults, published from September 1, 2018 until July 30, 2021. We included reviews, specifically to cover the time before 2018, as well as the primary literature, to cover more recent developments. All papers had to include protein biomarkers; we also list biomarkers of other types if these are mentioned alongside the proteins. All biomarkers had to

predict long-term (chronic, ≥3 months) outcome; their assignment to the categories of immune☐inflammation-related, coagulation-related, and adhesion-related was then based on the papers that report the markers, following the scheme by Lehmann *et al.* (2021). Finally, we used the STRING database (Jensen *et al.*, 2009) to exemplify a functional interaction network in which most of the protein biomarkers are involved. More specifically, we took all proteins from the table, submitted them to STRING, and captured the resulting network, as well as the gene ontology biological process annotation provided by STRING.

We first considered systematic reviews, from which we only transcribed the high-quality biomarkers into Table 1, that is, the biomarkers most consistently associated with outcome and (if available) with an added value compared to clinical routine (that is, compared to standard clinical and demographic markers that are routinely measured). We found no meta-analyses; this observation may be explained by the heterogeneity of the existing studies, according to Montellano *et al.* (2021). In fact, the review by Montellano *et al.* is the most exhaustive recent systematic review, based on 291 studies, screened until August 2018, including biomarkers measured up to 7 days post-stroke, and predicting outcomes thereafter. As the abstract states, "natriuretic peptides, copeptin, procalcitonin, mannose-binding lectin, adipocyte fatty acid–binding protein, and cortisol were the biomarkers most consistently associated with poor outcome in higher-quality studies showing an incremental value over established prognostic factors", where established factors refer clinical routine. These high-quality biomarkers we report in Table 1 (top), together with a few "atherogenesis" biomarkers highlighted by the same authors, reported by higher-quality studies to be associated with poor outcome (but with no explicit reference to any incremental/added value). The authors summarize the roles of the biomarkers as "inflammation, atherogenesis, and stress response", which overlaps with our classification of immune☐inflammation-related, coagulation-related, and adhesion-related biomarkers. Notably, they also report that CRP, TNFα and two cellular measurements (NLR (neutrophil-lymphocyte ratio) and WBC (white-blood cell count)) only feature inconsistent associations, while IL-6 and MMP-9 are only supported by one or two higher-quality studies of sufficient size, so these biomarkers could not meet the "*most consistently associated in higher-quality studies showing an incremental value*" criterion.

Donkel *et al.* (2019) provide another systematic review, based on 41 studies, screened until June 2018, with a focus on biomarkers of the hemostatic system (measured up until 72h post-event) as predictors, and disability (not considering death) as the outcome, and we list their results in Table 1 as well. More specifically, we went through their main table and transcribed biomarkers with evidence from more than one study, and where the majority of these studies had to demonstrate an association with outcome, but not necessarily as an association with an added value (i.e., the association did not have to be demonstrated in, e.g., a multivariable regression analysis). Study quality was reported in the supplement, but all studies that met the inclusion criteria were included, irrespective
of the quality score. The authors only consider biomarkers related to coagulation in the first place, without any further classification. Lai *et al.* (2019) also provide a systematic review, based on 18 studies, screened until September 2018, with a focus on biomarkers measured around 24h post-event, as predictors of (recovery of) physical function (not considering death) as the outcome; their biomarkers were included in Table 1 if they were reported to be "[c]onsistently [...] found to be robust predictors of long-term functional outcome in ischemic stroke" (Lai *et al.*, 2019). Study quality is assessed by Lai *et al.* (2019), but not used further in their analysis. The biomarkers are classified as related to immune response, lipids/metabolism

(no protein markers), neuronal function and blood vessel/circulation, again closely overlapping with our classification.

Next, we screened the primary literature from September 1, 2018 onwards, from which we transcribed biomarkers even if their added value was not established, into Table 1 (bottom). As noted by Montellano *et al.* (2021), few original studies consider the added value of the biomarkers they investigate, by reporting prediction models that consider clinical routine data as well (often, not even age, nor stroke severity are considered), and it appears that the situation has not changed much since the August 2018 cutoff of Montellano *et al.* (2021). Also, we did not formally assess study quality ourselves, and the overall number of participants is merely noted (though it is larger than 100 in all cases). Nevertheless, more recent studies usually feature more participants and, often, higher methodological quality, justifying the less strict criteria we imposed upon the recent primary literature.

In particular, from the study by Reiche *et al.* (2019), using the modified Rankin scale (mRS) as outcome, we only considered the predictor (not the association) analyses, based on logistic regression. We ignored the two composite scores reflecting inflammatory indices. The biomarkers were characterized as "immune-inflammatory, oxidative stress and biochemical". From the follow-up study by Alfieri *et al.* (2020), using 3-month mortality as outcome, we again only employed the predictor (not the association) analyses, although these included all variables without any adjustment, employing logistic regression. Among the four regression analyses presented, we took the one considering *all* biomarkers and the demographic/clinical data. Similarly to the first study, biomarkers were characterized as related to "immune-inflammatory, metabolic, oxidative, and nitrosative stress". In a third study by an overlapping set of authors, (Lehmann *et al.*, 2021), using 1-year mortality as outcome, the authors calculate predictors by logistic regression, neural network and support-vector-machine, and we took the consensus biomarkers as reported in the discussion section of the paper, considering these to be the most robust predictors. As already noted, biomarkers were classified as belonging to "immune☐inflammatory, coagulation and adhesion" (carotid intima-media thickness was not part of the consensus), and we adopted this scheme as the blueprint for our review, since these also fit well with the biological processes ascribed to the biomarkers found in the three systematic reviews.

We found a few more recent studies concerned with predicting outcome after stroke. Li *et al.* (2021) confirmed Lp-PLA2, which was already noted by Montellano *et al.* (2021). Finally, Li *et al.* (2019) investigated cytokine profiles, and found IL5 and IL6 as independent predictors of outcome.

Table 1. Blood-based biomarkers that predict stroke outcomes. Stringent criteria were applied to biomarkers reported in systematic reviews. HUGO gene names are given in parentheses where applicable. The color code of the biomarkers is red (immune-inflammatory), blue (coagulation-related) and magenta (adhesion-related). Non-protein biomarkers are given in italics. mRS: modified Rankin scale.

Referen	Biomarkers	Time	Other	Method /	Outcome	Number of

ce		measured, post-event	clinical variables considered / adjusted for	prediction algorithm	predicted	studies or partici-pants
Stroke outcomes, systematic reviews covering the time before September 2018						
Montellano *et al.* (2021)	*natriuretic peptides, copeptin, cortisol; procalcitonin;* mannose-binding lectin (MBL2); adipocyte fatty acid–binding protein (FABP4); *free fatty acids*, lipoprotein(a) (LPA), lipoprotein-associated phospholipase A2 (PLA2G7), osteoprotegerin (TNFRSF11B), *homocysteine; d-dimers*	up until 7 days	age and severity (in the majority of studies); etiology in a minority of studies	systematic review	functional outcome or mortality 7 days post-event or later	291 studies
Donkel *et al.* (2019)	Fibrinogen (FGA), *prothrombin fragment 1+2, d-dimers*, PAI-1 (SERPINE1)	up until 72h	various, depending on study	systematic review	disability score (Barthel Index or mRS)	41 studies
Lai *et al.* (2019)	CRP, IL6, TNFα (TNF)	around 24h	various, depending on study	systematic review	physical outcome	18 studies
Stroke outcomes, original papers since September 2018						
Lehmann *et al.* (2021)	IL6, TNFα (TNF), TGF-β1 (TGBB1), Protein S (PROS1), Protein C (PROC), VWF, *platelet count*, VCAM1, E-selectin (SELE)	within 24h	carotid intima-media thickness (cIMT)	consensus of logistic regression, neural network and support-vector-machine	mortality at 1 year	103 patients
Alfieri *et al.* (2020)	*systolic blood pressure, glucose, NOx, hydroperoxides,* IL6, *WBC, 25(OH)D*	within 24h	all variables included; sex is predictive as well	binary logistic regression	mRS at 3 months	176 patients 176 controls

Reiche *et al.* (2019)	IL6, ferritin (FTH1), *glucose, lipid hydroperoxides, 25(OH)D*	within 24h	not considered	binary logistic regression	mortality at 3 months	145 patients 176 controls
Li *et al.* (2021)	Lp-PLA2 (PLA2G7)				stroke recurrence	251 stroke patients 100 controls
Li *et al.* (2019)	IL5, IL6 (additionally, without adjustment: IL1β, IL4, IL7, IL9, IL10, IL15, *G-CSF, GM-CSF*)	within 24h	gender, age, smoking, alcohol abuse, hypertension, diabetes, hyperlipidemia, cardiovascular diseases, blood pressure	multivariate logistic regression analysis	mRS at 3 months	180 patients

Interaction network and gene ontology analysis of blood biomarkers of stroke corroborate their known roles

As described, the protein biomarkers for predicting outcome after ischemic stroke that we tabulated in this review (Table 1) can be assigned to the three classes of immune⬚inflammatory, coagulation-related, and adhesion-related proteins, following the scheme of Lehmann *et al.* (2021). The STRING functional interaction network assembled from these proteins (Fig. 1) groups them in the same way, placing immune⬚inflammatory proteins into the center of the network. PAI-1/SERPINE1 is given the role of a hub, reflecting its involvement in inflammation as well as coagulation (Hisada & Mackman, 2017; Valenzuela *et al.*, 2017). The inflammation-associated proteins IL6, CRP, TNF and TGFB1 are also given a central role. Non-protein biomarkers mentioned alongside the proteins in Table 1 refer to blood cell composition (platelets and white blood cells), glucose and lipids (free fatty acids and lipid hydroperoxides), remnants of coagulation processes (d-dimers, prothrombin fragment) (Friedmann *et al.*, 2019), as well as cardiac markers (natriuretic peptides, copeptin, blood pressure, relating to endothelial/vascular dysfunction) and general stress/health determinants (procalcitonin, cortisol, vitamin D). Most of these non-protein biomarkers are therefore also closely related to inflammation/immunity and to coagulation, and as future work we could envision a network that includes both protein and non-protein biomarkers, which may be based on publication-derived co-mentionings, or correlation data from longitudinal studies.

Our finding that ferritin (FTH1) was not connected in the network of blood-based biomarkers predictive of stroke outcomes deserves comment. In recent studies, ferritin in acute stroke patients was related to baseline rather than long-term disability (Reiche *et al.*, 2019; Alfieri *et al.*, 2020; Garg *et al.*, 2020). It has been shown in the animal model that increased body iron

indicated by ferritin worsens ischemic damage induced by transient ischemia and early reperfusion (Garcia-Yebenes *et al.*, 2012). Also in humans, increased serum ferritin was related to poor outcome after thrombolytic therapy in acute stroke due to early hemorrhagic transformation and severe brain edema (Millan *et al.*, 2007). Extracellular ferritin iron exacerbates the neurotoxic effect induced by glutamate excitotoxicity which plays a crucial role in acute brain ischemia (Millan *et al.*, 2008; Gamez *et al.*, 2021). Together with our STRING network analysis finding, we propose that ferritin mainly influences acute stroke severity, which is already reflected by clinical scores such as the NIHSS, and therefore does not add value in the prediction of long-term outcome.
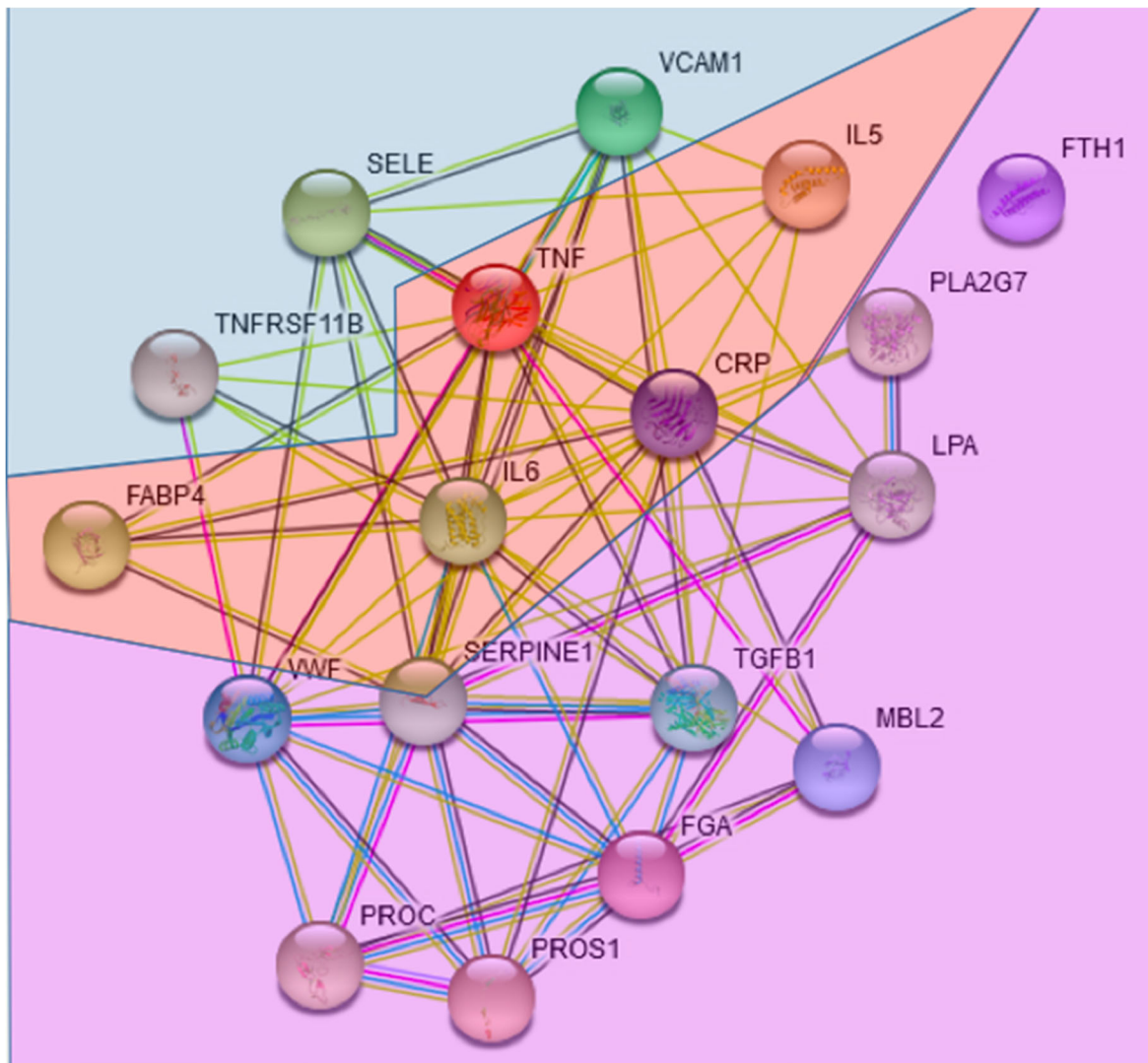
Fig. 1: STRING network of the proteins in Table 1. The three adhesion-related biomarkers are positioned at the top; the coagulation-related biomarkers are positioned at the bottom. The immune-inflammation biomarkers are found in the middle of the network (pale red). Even though TNFRSF11B is not easy to classify, it increases leukocyte adhesion to endothelial cells (Zauli *et al.*, 2007), rendering it an adhesion-related protein. Only FTH is not connected in the STRING-based network, using default parameters.

Table 2: Gene ontology biological process enrichment (first 50 terms) provided by STRING for the network of Fig. 1. The color code of the biomarkers is red (immune-inflammatory), blue (coagulation-related) and magenta (adhesion-related). The pale red color refers to immune-inflammatory processes in a wider sense. FDR: false discovery rate.

term description	observed gene count	background gene count	FDR	matching proteins in network (labels)
response to bacterium	10	555	1.17E-08	TGFB1,SERPINE1,CRP,VCAM1,TNFRSF11B,FGA,SELE,MBL2,IL6,TNF
extracellular structure organization	9	339	1.17E-08	TGFB1,SERPINE1,VWF,PLA2G7,VCAM1,TNFRSF11B,FGA,LPA,TNF
inflammatory response	9	482	8.37E-08	TGFB1,IL5,CRP,VCAM1,TNFRSF11B,SELE,MBL2,IL6,TNF
regulation of inflammatory response	8	338	1.42E-07	SERPINE1,PROC,FABP4,PLA2G7,SELE,PROS1,IL6,TNF
defense response	11	1234	4.79E-07	TGFB1,SERPINE1,IL5,CRP,VCAM1,TNFRSF11B,FGA,SELE,MBL2,IL6,TNF
regulation of defense response	9	676	7.10E-07	SERPINE1,PROC,FABP4,PLA2G7,FGA,SELE,PROS1,IL6,TNF
regulation of response to external stimulus	10	955	7.10E-07	TGFB1,SERPINE1,PROC,FABP4,PLA2G7,FGA,SELE,PROS1,IL6,TNF
extracellular matrix organization	7	296	1.18E-06	TGFB1,SERPINE1,VWF,VCAM1,TNFRSF11B,FGA,TNF
response to lipopolysaccharide	7	298	1.18E-06	TGFB1,SERPINE1,VCAM1,TNFRSF11B,SELE,IL6,TNF
positive regulation of mononuclear cell migration	4	25	1.84E-06	TGFB1,SERPINE1,PLA2G7,TNF
response to stress	14	3267	2.58E-06	TGFB1,SERPINE1,IL5,PROC,CRP,VWF,VCAM1,TNFRSF11B,FGA,SELE,MBL2,PROS1,IL6,TNF
positive regulation of immune system process	9	882	2.92E-06	TGFB1,SERPINE1,IL5,PLA2G7,VCAM1,FGA,MBL2,IL6,TNF
immune system process	12	2370	7.81E-06	TGFB1,IL5,CRP,FTH1,VCAM1,TNFRSF11B,FGA,SELE,MBL2,PROS1,IL6,TNF
regulation of immune system process	10	1391	7.81E-06	TGFB1,SERPINE1,IL5,PLA2G7,VCAM1,FGA,MBL2,PROS1,IL6,TNF
defense response to bacterium	6	250	7.81E-06	SERPINE1,CRP,FGA,MBL2,IL6,TNF
positive regulation of inflammatory response	5	120	7.81E-06	SERPINE1,FABP4,PLA2G7,IL6,TNF
positive regulation of leukocyte migration	5	127	8.11E-06	TGFB1,SERPINE1,PLA2G7,IL6,TNF
platelet degranulation	5	129	8.37E-06	TGFB1,SERPINE1,VWF,FGA,PROS1

negative regulation of blood coagulation	4	48	9.50E-06	SERPINE1,PROC,FGA,PROS1
positive regulation of response to external stimulus	7	499	1.18E-05	TGFB1,SERPINE1,FABP4,PLA2G7,FGA,IL6,TNF
regulation of response to stimulus	14	3882	1.25E-05	TGFB1,SERPINE1,IL5,PROC,FABP4,PLA2G7,VCAM1,TNFRSF11B,FGA,SELE,MBL2,PROS1,IL6,TNF
leukocyte migration	6	296	1.25E-05	TGFB1,VCAM1,SELE,PROS1,IL6,TNF
regulation of immunoglobulin production	4	62	2.01E-05	TGFB1,IL5,IL6,TNF
defense response to other organism	8	859	2.35E-05	TGFB1,SERPINE1,CRP,VCAM1,FGA,MBL2,IL6,TNF
positive regulation of transport	8	892	2.85E-05	TGFB1,SERPINE1,IL5,FGA,SELE,MBL2,IL6,TNF
acute inflammatory response	4	73	3.21E-05	CRP,VCAM1,MBL2,IL6
positive regulation of defense response	6	365	3.27E-05	SERPINE1,FABP4,PLA2G7,FGA,IL6,TNF
negative regulation of lipid storage	3	17	3.38E-05	CRP,IL6,TNF
leukocyte tethering or rolling	3	17	3.38E-05	VCAM1,SELE,TNF
regulation of immunoglobulin secretion	3	17	3.38E-05	IL5,IL6,TNF
localization	15	5233	3.46E-05	TGFB1,SERPINE1,PROC,CRP,FABP4,VWF,FTH1,VCAM1,FGA,LPA,SELE,MBL2,PROS1,IL6,TNF
fibrinolysis	3	21	4.81E-05	SERPINE1,FGA,PROS1
response to tumor necrosis factor	5	217	4.91E-05	FABP4,VCAM1,TNFRSF11B,SELE,TNF
regulation of protein secretion	6	422	5.53E-05	TGFB1,IL5,CRP,FGA,IL6,TNF
response to cytokine	8	1035	5.92E-05	TGFB1,IL5,FABP4,VCAM1,TNFRSF11B,SELE,IL6,TNF
regulation of endocytosis	5	229	5.99E-05	TGFB1,SERPINE1,SELE,MBL2,TNF
positive regulation of cell-cell adhesion	5	238	7.07E-05	TGFB1,VCAM1,FGA,IL6,TNF
positive regulation of protein secretion	5	240	7.22E-05	TGFB1,IL5,FGA,IL6,TNF

negative regulation of protein metabolic process	8	1075	7.28E-05	TGFB1,SERPINE1,FABP4,FGA,LPA,PROS1,IL6,TNF
wound healing	6	461	8.02E-05	TGFB1,PROC,VWF,FGA,PROS1,IL6
negative regulation of multicellular organismal process	8	1098	8.08E-05	TGFB1,SERPINE1,PROC,TNFRSF11B,FGA,PROS1,IL6,TNF
immune response	9	1560	9.69E-05	TGFB1,IL5,FTH1,VCAM1,TNFRSF11B,FGA,MBL2,IL6,TNF
defense response to Gram-positive bacterium	4	111	9.69E-05	CRP,MBL2,IL6,TNF
regulation of body fluid levels	6	483	9.69E-05	SERPINE1,PROC,VWF,FGA,PROS1,IL6
regulation of vesicle-mediated transport	6	480	9.69E-05	TGFB1,SERPINE1,FGA,SELE,MBL2,TNF
regulation of superoxide metabolic process	3	33	0.00012	TGFB1,CRP,TNF
transport	13	4130	0.00013	TGFB1,SERPINE1,PROC,CRP,FABP4,VWF,FTH1,FGA,LPA, MBL2,PROS1,IL6,TNF
blood coagulation	5	288	0.00013	PROC,VWF,FGA,PROS1,IL6
positive regulation of endocytosis	4	129	0.00014	SERPINE1,SELE,MBL2,TNF
vesicle-mediated transport	9	1699	0.00016	TGFB1,SERPINE1,PROC,CRP,VWF,FTH1,FGA,MBL2,PROS1

Blood biomarkers of stroke overlap with biomarkers of cellular senescence, thromboembolism and sarcopenia, with links to cancer

Given the classification of the biomarkers we presented, it is of interest whether alternative classifications, relating to other biological processes, may be possible. On one hand, the GO annotations in Table 2 further refer to lipid storage (supported only by immuno-inflammatory proteins, though), protein secretion / endocytosis, wound healing, and some other general metabolic processes. On the other hand, we may consider more general biological processes such as sarcopenia, and overlapping biological processes such as cellular senescence (Mankhong *et al.*, 2020). The latter is not frequently mentioned in the literature on predicting ischemic stroke outcome, though there are exceptions (Childs *et al.*, 2017; Valenzuela *et al.*, 2017). Our analysis corroborates the evidence for its role in the natural history of stroke-related health deterioration, given that many members of the SASP (senescence-associated secretory

phenotype; SERPINE1, IL6, CRP, TNF and TGFB1) are placed center stage in the interaction network. At the minimum, we suggest that the inflammatory processes taking place after stroke are expected to be pushed further by senescent cells secreting just the factors that predict health deterioration after stroke. As we suggested elsewhere, cellular senescence may indeed be a driver of the co-morbidity of stroke and (pancreatic) cancer, including thromboembolic events (Henze *et al.*, 2020).

In fact, thromboembolism biomarkers in patients with cancer were reviewed recently (Gervaso *et al.*, 2021) and apart from platelet and leukocyte counts, the authors list tissue factor (Coagulation Factor III), d-dimers, soluble P-selectin and CRP, overlapping with the markers of Table 1. While these biomarkers refer to venous thromboembolism, data on biomarkers for arterial thrombotic events specifically in cancer patients are sparse (Gervaso *et al.*, 2021). Very recently, a prospective study described biomarkers in cancer patients with stroke (not necessarily predictive of future outcomes), in stroke patients that do not feature cancer as a comorbidity, and it also contrasts these with another group of patients that are only affected by cancer (Navi *et al.*, 2021); each patient group had size 50. Interestingly, most association biomarkers are valid for stroke patients no matter whether they are also affected by cancer; these are d-dimer, ICAM-1, VCAM-1, thrombomodulin. Only one biomarker (P-selectin) is found associated exclusively in the stroke-only group, and thrombin-antithrombin is found associated exclusively in the cancer-only group. Here, biomarkers were measured around 96h after the event/diagnosis; multivariable linear regression was used to reveal associations to an outcome defined by prespecified hematological biomarkers and transcranial Doppler microemboli detection, adjusted for race, number of stroke risk factors, smoking, antithrombotics use, and NIHSS. Of interest, all biomarkers of Navi *et al.* (2021) are related to coagulation or cell adhesion, and we also note the overlap with the biomarkers of Table 1.

Finally, the discrimination between 100 sarcopenia patients and 100 controls was recently demonstrated by Marzetti *et al.* (2019), based on the proteins MPO, P-selectin, IL8, MCP-1, CRP, MIP-1α, PDGF-BB, and IP-10. Again, there is an overlap with our biomarkers, supporting the hypothesis that thrombotic events, including recurrent stroke, as well as sarcopenia may be triggered by post-stroke coagulation and inflammation. A recent transcriptomics analysis highlights the role of the aging immune system in acute stroke (Sykes *et al.*, 2021) and suggests that not just the inflammatory aspects of the immune system play a role in determining disease outcome. However, the functional status of the (adaptive) immune system is not as easy to measure as its contribution to inflammatory processes, explaining why no such markers are prominently reported as protein biomarkers in the blood. Moreover, immunity and inflammation are closely connected in general, and even more so in stroke, considering the immune-cell infiltration that is triggered by stroke.

As the major limitation of any study of this kind, we are limited to the small sets of biomarkers measured a-priori in any of the studies performed thus far, resulting in inspector bias effects. Such a bias may be alleviated by high-throughput omics studies considering transcripts, proteins, lipids or other metabolites. However, transcripts are known to be quite noisy, large-scale proteomics is lacking, and metabolites are not as straightforward in their assignment to biological processes, even though in this review, we consider some of these alongside the proteins. Nevertheless, the most recent transcriptomic analysis (Sykes *et al.*, 2021) highlighted immune-related processes that are closely associated with the observations presented in this review, including interleukin signalling, though on the gene level, they found no transcripts that

directly correspond to any of the protein biomarkers in Table 1.

Overall, we suggest that our summary of the outcome biomarkers for ischemic stroke provides strong evidence for the role of immune☐inflammatory, coagulation-related, and adhesion-related processes in the health deterioration and mortality after stroke. The overlap of the outcome biomarkers with the SASP and with thromboembolism biomarkers, as well as the role of senescent cells in blood clotting (Tanaka *et al.*, 2018), suggests that cellular senescence plays a role as well. Cellular senescence is also implicated in sarcopenia (Mankhong *et al.*, 2020), and we found an overlap of our outcome biomarkers for stroke with sarcopenia biomarkers, suggesting further that sarcopenia is a specific form of health deterioration that may also be co-driven by cellular senescence.

Conflict of Interest:

UW reports grants and personal fees from Merz Pharma, and personal fees from Allergan, Ipsen Pharma, Bristol-Myers Squibb, Daiichi Sankyo, Bayer Vital, Boehringer Ingelheim, Pfizer, Thieme, and Elsevier Press, all outside the submitted work. The other authors have nothing to disclose.

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