Characterization of cerebral small vessel disease by neutrophil and platelet activation markers using artificial intelligence

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Debette and Markus, 2010; Wardlaw et al., 2019). The most common type of cSVD is age- and hypertension-related sporadic SVD. In current clinical practice, magnetic resonance imaging (MRI) is used for diagnosis. The occurrence of pathologic MRI features, e.g. white matter hyperintensities (WMH), lacunar infarcts, microbleeds, perivascular spaces, and cerebral atrophy is common in cSVD patients and considered diagnostic for cSVD (Wardlaw et al., 2013). There is no causative treatment with proven efficacy against cSVD and current therapeutic
options are limited to general cardiovascular risk management like lowering blood pressure and plasma cholesterol, and antiplatelet therapy. Despite advances during the last decades in the field of neuroimaging and biomarkers, the pathogenesis of cSVD is still poorly characterized.

Although pathologic features typical for cSVD have been identified, the definition of distinct pathologic stages for cSVD is still challenging (Mustapha et al., 2019). It is hypothesized that a loss of the ability to regulate cerebral blood flow in response to variations in blood pressure during ageing may initiate its development. In addition, hypertension and increased arterial stiffness may result in increased blood flow velocities and increased pulsatility in the cerebral arteries. These hemodynamic changes might subsequently lead to dysfunctional cerebral microvascular endothelium, disrupting intercellular communication with perivascular cells and oligodendrocytes or their precursors (Mustapha et al., 2019) and leading to an alteration of blood brain barrier (BBB) integrity and permeability (Zhang et al., 2017). There is increasing evidence that endothelial dysfunction and BBB leakage are involved in cSVD pathophysiology (Cuadrado-Godia et al., 2018; Skoog et al., 1998; Wardlaw et al., 2005; Zhang et al., 2017), which is supported by circulating biomarkers of endothelial dysfunction, e.g. soluble forms of ICAM-1, VCAM1, CD62E and CD62P (Poggesi et al., 2016; Rouhi et al., 2012). Next to endothelial dysfunction, inflammatory responses and leukocyte infiltration are common pathological features of cSVD and may also contribute to, or further propagate its pathogenesis (Fu and Yan, 2018; Koizumi et al., 2019; Low et al., 2019).

Previous studies have shown a relation between endothelial cell activation and endothelial dysfunction in patients with WMH and lacunar infarction (de Leeuw et al., 2002; Fassbender et al., 1999; Fornage et al., 2008; Hassan et al., 2003). During the past years, a possible role of neutrophils in cSVD is gaining increased attention. For example, the neutrophil to lymphocyte ratio, a marker of systemic inflammation, was found to be associated with WHM in healthy people (Nam et al., 2017). Neutrophil and NETs (nuclear DNA expelled by neutrophils) contain many cytotoxic and inflammatory compounds, with the potential to induce endothelial activation and damage (Xu et al., 2009), the production of the cytokine interleukin-1 (Folco et al., 2018), and to cause ischemic brain injury and BBB disruption (Armao et al., 1997; Segel et al., 2011). Thus, markers of neutrophil activation such as free or DNA-associated myeloperoxidase (MPO) and calprotectin (S100A8/A9) might help unravel a part of the pathogenesis of cSVD and might offer possibilities for identification and classification of individuals with this illness.

The vasculature is in tight contact with platelets. Platelets rapidly respond to endothelial cell activation and inducible nitric oxide synthase (iNOS) in patients with WMH and lacunar infarction (Coenen et al., 2014). In addition, CXCL1 (fractalkine) and interleukin 1β (IL-1β) were measured as markers of vascular inflammation (Folco et al., 2018; Hildemann et al., 2014; Hundhausen et al., 2003; Ludwig et al., 2002). To address neutrophil activation and NETs, the markers MPO (myeloperoxidase), free or in complex with DNA (MPO-DNA), and S100A8/A9 (calprotectin) were measured. The concentrations of CXCL1, CXCL4, CXCL7, MPO, and S100A8/A9 were measured using specific human DuoSet ELISA assays (R&D Systems, Minneapolis, MN), except IL-1β (Thermo Fisher Scientific, Waltham, MA). In addition, the MPO DuoSet ELISA assay was used with picogreen dsDNA (QuantiTm PicoGreen® dsDNA Assay Kit, Thermo) to measure MPO-DNA as previously described (Hally et al., 2021; Jimenez-Alcalar et al., 2018). All ELISA assays were conducted in duplicate according to manufacturer’s instructions, except that recommended sample volumes were divided by two. The concentrations of CXCL1 and IL-1β were measured in undiluted plasma, MPO in 30- and 60-fold dilutions, CXCL4 in 1000- and 1500-fold dilutions and CXCL7 3500- and 4000-fold dilutions. Phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) was used as the reagent diluent. Optical density (OD) of samples were read at 450 nm and wavelength correction was set to 540 nm, using an EL808 Ultra Microplate Reader and Gen5 software (Bio-Tek Instruments, Winooski, VT). Standard curves and sample concentrations were calculated using a four-parameter logistic regression algorithm in.
Python 3. MPO-DNA was measured in 96-well plates using 3 flashes per well for measuring fluorescence at 480 nm excitation and 520 nm emission using FlexStation 3 (Molecular Devices, San Jose, CA). ELISA measurements of all patient and matching control samples were performed using the same assay, simultaneously and in the same institution.

2.4. Machine learning

Python machine learning library Scikit-learn (open-source software) was used to perform regularized logistic regression (Pedregosa et al., 2011). Liblinear library was used as solver in the regularized logistic regression to determine optimal fit for categorized data (scikit-learn, 2021). In total 67% of the cases were used to train the regularized logistic regression and 33% were used to validate the model. Random forest, also known as random decision forest, uses random sampling and features (including biomarkers, demographics and clinical characteristics of patients and controls) to generate multiple decision trees (n = 100). In total 80% of the data was used to generate 100 different decision trees to generate one classification algorithm by a forest of decision trees. K-Nearest Neighbor (KNN) mean is a supervised classification algorithm that requires labelled data, to obtain new data points accordingly to the k number of the closest data points. KNN algorithm assumes that similar subjects, in this study individuals with or without cSVD, exist in close proximity. For regularized logistic regression and KNN means normalized data was entered. As random forest uses Euclidean distance in an n-dimensional space, categorized data was used to perform regularized logistic regression and KNN. In total 67% of the cases were used to train the random forest and 33% were used to validate the model.

Accuracy = \frac{100\% \times \text{True Positive} + \text{True Negative}}{\text{True Positive} + \text{False Positive} + \text{True Negative} + \text{False Negative}}.

Precision = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}.

Sensitivity = \frac{100\% \times \text{True Positive}}{\text{True Positive} + \text{False Negative}}.

Specificity = \frac{100\% \times \text{True Negative}}{\text{True Negative} + \text{False Positive}}.

In which true positive is the number of cases correctly identified as patient, false positive is the number of cases incorrectly identified as patient, true negative is the number of cases correctly identified as healthy and false negative is the number of cases incorrectly identified as healthy.

2.5. Statistics

Statistical analyses were performed with GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) or in R 4.1.0 (CoreTeam, 2021). Data were checked for normality with the D’Agostino Pearson omnibus normality test. All continuous data are presented as median (interquartile range, IQR). Categorical data were displayed as frequency (percentage). Significance of differences were determined by Mann-Whitney U test, Kruskal Wallis, or one-way ANOVA, as appropriate. P values <0.05 were considered to be significant.

3. Results

3.1. Study population

Blood was collected from 80 cSVD patients and 38 healthy sex- and age-matched individuals (Table 1) (Zhang et al., 2017). The majority of cSVD patients (57.5%) were male, had a median age of 72.5 (63.3–78.0) years. Hypertension (63.8%), hypercholesterolemia (56.3%), smoking (23.4%), and statin use (62.5%) were overrepresented in the cSVD group, compared with controls (47.4%, p = 0.037; 34.2%, p = 0.025; 7.89%, p = 0.039; 31.6%, p = 0.002, respectively). Among the cSVD patients, 42.5% had one or more lacunes on MRI versus 7.9% in the control group, and WMH volume was higher (both p < 0.0001). Additional characteristics of patients diagnosed with first-ever lacunar stroke (n = 44, Laci) and with mild vascular cognitive impairment (n = 36, mVCI) are given in Suppl. Table 1.

Table 1

| Characteristics of cSVD patients and healthy controls. |
|-----------------|-----------------|
| cSVD (n = 80) | Control (n = 38) |
| Age (years) | 72.5 (63.8–78.0) | 71.0 (63.3–79.0) |
| Sex, male, n (%) | 46 (57.5) | 23 (60.5) |
| BMI kg/m² (IQR) | 25.5 (22.6–27.4) | 26.3 (24.4–29.0) |
| Coronary artery disease n (%) | 15 (18.9) | 4 (10.5) |
| Hypertension n (%) | 51 (63.8)* | 18 (47.4) |
| Diabetes n (%) | 12 (15.0) | 4 (10.5) |
| Hypercholesterolemia n (%) | 45 (56.3)* | 13 (34.2) |
| Peripheral arterial disease n (%) | 5 (6.25) | 1 (2.63) |
| Smoking n (%) | 19 (23.4)* | 3 (7.89) |
| Statin use n (%) | 50 (62.5)** | 12 (31.6) |

Data shown as median (IQR) or n (%), * p < 0.05, ** p < 0.1, # p < 0.0001, cSVD vs Control, Mann-Whitney test.

3.2. Levels of individual biomarkers within the study cohort

Platelet activation biomarkers CXCL4 and CXCL7, vascular inflammation markers CX3CL1 and IL-1β and markers of neutrophil activation and NETs MPO, MPO-DNA and S100A8/A9 were measured in plasma from patients and controls, and directly compared by univariable analysis (Fig. 1, Table 2). While all IL-1β levels were below detection limits, the concentrations of CXCL4, CXCL7, CX3CL1 and S100A8/A9 did not significantly differ between patients and controls before (Fig. 1, Table 2), or after stratification of the patients for mVCI and Laci (Suppl. Fig. 1). Some CX3CL1 concentrations were below detection limits and most were found to be in the pg/mL range, which is low but in agreement with previous findings (Damas et al., 2005; Flierl et al., 2015). Stratification revealed a tendency towards different levels of CX3CL1 in mVCI and Laci, but the difference was not statistically significant (p = 0.066) (Fig. 2A). MPO-DNA, which reflects NET-release also did not differ between cSVD patients and controls, without (Fig. 1, Table 2) or with stratification (Suppl. Fig. 1 and suppl. Table 2).

MPO levels were significantly increased in cSVD compared to controls (Fig. 1, Table 2). When the cSVD group was split into mVCI and Laci, the MPO levels were significantly increased in the Laci, but not the mVCI group, when compared to controls (Fig. 2B and Suppl. Table 2). A multivariable linear regression analysis indicated that MPO levels were not confounded by overrepresented patient characteristics.
(hypertension, hypercholesterolemia, smoking, and statins, Table 1) or by diabetes (Suppl. Table 3).

3.3. Composition of multivariate models for machine learning analysis

As MPO was the only plasma marker that was elevated in cSVD patients, we aimed to determine whether discrimination between cSVD and controls could be improved by incorporating demographics and clinical and MRI characteristics of both patients and controls. For this analysis only patients and controls with a complete dataset were included, leading to the exclusion of 4 cSVD patients and a final inclusion of 76 cSVD patients and 38 controls into the analysis.

Three different machine learning algorithms were implemented: 1) regularized logistic regression, 2) KNN means, and 3) random forest. cSVD/control was set as the dependent variable, and all 28 patient characteristics, measurements and markers were considered independent variables for the 3 models.

Four different models were composed and compared to each other: A) patient characteristics alone, B) patient characteristics were combined with MRI characteristics, which is the state-of-the-art for diagnosis.
of cSVD patients, C) patient characteristics, without MRI characteristics, with platelet, neutrophil and vascular plasma markers, D) patient characteristics, with MRI, with platelet, neutrophil and vascular plasma markers (all parameters). The individual parameters are listed in Suppl. Table 4.

3.4. Regularized logistic regression

The results from regularized logistic regression are visualized in Fig. 3 and summarized in Table 3. Model accuracy, specificity and sensitivity, as well as receiver operating characteristic (ROC) curves (insets in Fig. 3A-D) and the corresponding area under the curve (AUROC) were calculated (Table 3). Entering only patient characteristics (model A) included 9 out of the 17 possible parameters in an optimized predictive model, listed in Fig. 3A (e.g. LDL levels, diabetes, hypertension, BMI, and smoking), with an accuracy of 56.4% and an AUROC of 52.7 (Table 3), indicating poor predictive performance of this model. The prediction improved in model B in which MRI characteristics (periventricular and/or deep extensive white matter hyperintensities, lacunar lesions, and deep perivascular spaces) were entered in addition to the patient characteristics (model accuracy: 66.7%; AUROC: 62.0, Table 3), indicating robust predictive performance of this model. The prediction improved in model B in which MRI characteristics were incorporated (model B), patient characteristics remained the most important features in the generation of the decision trees (Fig. 3B). When MRI parameters were replaced by the ELISA plasma markers (model C), MPO and LDL remained the most important features (AUC = 0.842, Table 3), indicating robust predictive performance. In retrospect, the inclusion of the plasma marker data appeared to compensate for the exclusion of MRI characteristics resulting in a model with comparable predictive quality, when considering the AUROC values (Table 3, model C vs model B). Model D also showed highest predictive potential when a KNN algorithm was implemented, while the models A-C only showed poor prediction using this algorithm (Suppl. Table 5).

3.5. Random forest analysis

To further support the above results and to identify additional relevant parameters associated with cSVD, a random forest analysis was performed. In this analysis, 100 decision trees were generated obtaining a so-called forest in which each parameter is scaled to their importance. The feature importance of all individual parameters sums up to 1. The top 10 most important parameters of each model are listed in the respective panels A-D of Fig. 4. Model accuracy, sensitivity, specificity and accompanying out-of-bag (OOB) scores are shown in Table 4. The OOB score reflects how well those subjects who were left out of the sample during bootstrapping, were predicted by the algorithm during the training of the random forest model. The model score reflects how well the subjects of the test dataset (i.e. the 20% random sample) were predicted by the random forest algorithm after completion of the training with the remaining 80% of the dataset. Whereas the regularized logistic regression algorithm resulted in models that showed a high specificity in distinguishing cSVD patients from controls but were less efficient in detecting cSVD (sensitivity, Table 3), the random forest algorithm rather resulted in models that could sensitively detect cSVD yet poorly distinguished patients from controls (low specificity) (Table 4). Thus, the primary utility of the random forest algorithm was to identify potential predictors of cSVD among the parameters investigated.

In accordance with the regularized logistic regression analysis, model A, which includes only patient/control characteristics again returned LDL and BMI as important parameters, and also returned systolic blood pressure, age, HDL, triglycerides, creatinine and glucose levels (Fig. 4A). Interestingly, even when MRI parameters were incorporated (model B), patient characteristics remained the most important features in the generation of the decision trees (Fig. 4B). When MRI parameters were replaced by the ELISA plasma markers (model C), MPO and LDL were returned as the most important features, followed by MPO-DNA, S100A8/A9 and CXCL4 (Fig. 4C). When all parameters were entered (model D), LDL and MPO remained the most important features,
Fig. 3. Graphical results of regularized logistic regression of cSVD and control characteristics.

A) Regularized logistic regression analysis of model A, the dataset of patient characteristics, resulted in the selection of: LDL/HDL, low / high density lipoprotein; hypercholesteremia; DM, diabetes mellitus; hypertension; DBP, diastolic blood pressure; BMI, body mass index; smoking; and sex, by the algorithm.

B) Regularized logistic regression analysis of model B, including patient and MRI parameters, resulted in the selection of: WMH PVD, periventricular and/or deep extensive white matter hyperintensities; presence of lacunes; and PVS D, deep perivascular spaces, by the algorithm.

C) Regularized logistic regression analysis of model C, patient characteristics and plasma marker data, resulted in the selection of: LDL; hypercholesteremia; DBP; hypertension; and MPO, Myeloperoxidase, by the algorithm.

D) Regularized logistic regression analysis of model D, including the entire set of variables, resulted in the selection of: WMH PVD; PVS D; lacunar infarct; LDL; hypercholesteremia; MPO; hypertension; number of microbleeds; BMI; CXCL7; and HDL, by the algorithm.

Bar graphs represent p-values of the F-statistic for the features which have the most predictive power for each dataset. Inset: ROC curve of true positive rate (TPR) versus false positive rate (FPR). Analysis was performed on data from 76 cSVD patients and 38 controls (training data n = 76, validation data n = 38).

Table 3
Characteristics of optimal prediction model for each dataset.

| Multivariate model | Characteristics multivariate model |
|--------------------|-----------------------------------|
| Patient char. | MRI char. | ELISA markers | N param. | AUROC | Accuracy (%) | Sensitivity (%) | Specificity (%) |
| A | + | – | – | 9/17 | 52.7 | 56.4 | 23.5 | 81.8 |
| B | + | + | – | 3/22 | 62.0 | 66.7 | 50 | 74.1 |
| C | + | – | + | 5/23 | 63.2 | 71.3 | 38.5 | 88.0 |
| D | + | + | + | 11/28 | 73.4 | 73.7 | 72.7 | 74.1 |

Logistic regression analysis of a dataset returns a prediction with a number of features, selected from a maximum of 28 features, depending on the model. AUROC: area under receiver operating characteristic curve.
followed by the MRI parameter deep perivascular spaces (Fig. 4D). Although their concentrations were not significantly different between control and cSVD groups (Fig. 3), MPO-DNA, CXCL4 and S100A8/A9 were nevertheless returned as important features in predicting whether a person has cSVD or not (Fig. 4D).

4. Discussion

In the present study, plasma markers of vascular inflammation, platelet and neutrophil activation were measured in a cohort of patients with cSVD and age-matched controls. In addition, machine learning technology was implemented in order to identify whether these markers

![Fig. 4. Graphical results of random forest analysis of cSVD and control characteristics.](image)

**Table 4**

Characteristics of random forest for each dataset.

| Model | Patient char. | MRI char. | ELISA markers | Score | OOB score | Accuracy (%) | Sensitivity (%) | Specificity (%) |
|-------|---------------|-----------|---------------|-------|-----------|--------------|----------------|----------------|
| A     | +             | −         | −             | 0.65  | 65.2      | 68.8         | 57.1           |
| B     | +             | +         | −             | 0.82  | 73.9      | 93.8         | 28.6           |
| C     | +             | −         | +             | 0.68  | 69.6      | 87.5         | 28.6           |
| D     | +             | +         | +             | 0.76  | 78.3      | 100          | 28.6           |

Sensitivity and specificity were calculated from the prediction results of the test dataset.
could distinguish controls from patients, thereby providing additional clues about cSVD aetiology.

Of the plasma markers measured, only MPO levels were significantly elevated in patients with cSVD in a direct comparison with age- and sex-matched controls. After patient stratification, increased MPO levels were found to be specifically associated with lacunar stroke. The MPO levels were not confounded by diabetes or overrepresented features in the patient group. MPO is stored in the primary secretory granules of neutrophils (Nauseef, 2014). MPO has a cytotoxic effector function as an enzymatic source of hypochlorite, important for the elimination of invading pathogens, and is also required for the proper release of NETs (Papayannopoulos et al., 2010). Although MPO antigen levels indirectly imply MPO activity (Pulli et al., 2013), circulating MPO levels have been in focus as a biomarker for inflammation, mainly reflecting the activation of neutrophils (Fuchs et al., 2012). In addition, the circulating complex of DNA with MPO serves as a biomarker for the release of NETs (Fuchs et al., 2012; Hally et al., 2021; Jimenez-Alcazar et al., 2018; Konkoth et al., 2021). Our finding that increased MPO levels were specifically associated with lacunar stroke suggests that neutrophil activation might be involved in the pathophysiology of lacunar infarct. Moreover, it is conceivable that tissue damage can be exacerbated by the reactive oxygen species generated by MPO, a process that is implicated to drive the progression of neurodegenerative disease (Gellhaar et al., 2017; Ray and Katyal, 2016). In addition, mice lacking MPO were protected against the progression of cognitive decline in a model of Alzheimer’s disease (Volkman et al., 2019) and polymorphisms in the MPO gene were found to modify the risk of Alzheimer’s disease in a population of Han Chinese (Ji and Zhang, 2017). Besides its cytotoxic effector function as an enzymatic source of hypochlorite, important for the elimination of invading pathogens, MPO is also required for the proper release of NETs (Papayannopoulos et al., 2010). A recent study indicates that dietary supplementation with anserine, a scavenger of hypochlorite, slowed the progression of cognitive decline in a cohort of patients with cSVD (Hildemann et al., 2014; Konkoth et al., 2021; Postea et al., 2012; Schafer et al., 2004; Schulz et al., 2007). The observation that the levels of CxCL1 between patients and controls were not different in this study suggests that cSVD is not accompanied by an increased inflammatory activation of CxCL1-expressing cells, or by inflammation in general. This notion is supported by a recent study in patients with cSVD, that did not reveal differences in IL-1β and TNFα levels between patients and controls (Wang et al., 2020). However, CxCL1 measurements might be interesting for the characterization of microglia in future studies, as CxCL1 concentrations showed a trend towards elevation in the microglia group. Interestingly, the levels of the neutrophil attractant CXC8 were significantly elevated in patients with cSVD and found to be associated with chronic insomnia (Wang et al., 2020). Of note, the chemokine CXCL8 and CXCL7 both activate neutrophils by the receptor CXCR2 (Russo et al., 2014) and activation of CXCR2 induces the release of NETs, for example during thrombosis (Teijeira et al., 2021; Vago et al., 2018).

To further explore the relevance of the plasma markers determined in this study, machine learning algorithms were implemented. Interestingly, both the regularized logistic regression and random forest algorithms returned plasma LDL levels as a strong predictor of cSVD. In the regression-based machine learning algorithm, patient and MRI characteristics dominated over the ELISA plasma markers as predictors, with only MPO and CXCL7 returned as possible predictors. At the level of prediction accuracy, the inclusion of patient characteristics alone or combined with the plasma markers led to >80% specificity in distinguishing cSVD patients from controls, but in poor sensitivity (<40%) for identifying cSVD. Inclusion of all parameters in the model improved the AUROC, and thus the general quality of prediction. Interestingly, the decision tree-based random forest algorithm had a higher preference for the ELISA plasma markers than the regularized logistic regression algorithm, suggesting that these markers facilitated cut-off point selection during the generation of decision trees. Using random forest, patient characteristics dominated over MRI parameters when plasma markers were not included. When included in the model, MPO was consistently returned as the most important feature, along with LDL levels. Also CXCL4, MPO-DNA and S100A8/A9 were among the top 10 most important features, despite the lack of significant differences between their concentrations between the cSVD and control groups. At the level of prediction accuracy however, all random forest-based models were able to accurately detect cSVD in the cohort, with sensitivities of up to 100%, but performed poorly in distinguishing controls from cSVD patients. The predictions were not improved by the implementation of a third KNN algorithm. Taken together, the machine learning algorithms do provide information about possible pathophysiologic determinants of cSVD and yet are currently not optimal to generate models that allow accurate detection of cSVD or to distinguish non-SVD from cSVD individuals.

The study has some limitations, for example the number of subjects included might not be optimal to unfold the full potential of the machine learning algorithms. In addition, the ratio of 2 cSVD patients to 1 control might not be sufficient for the algorithms to perform well. Finally, several basic features, e.g. hypercholesteremia and smoking, were inherently different between the cSVD and control groups, which might skew the learning process in the machine learning algorithms. Although this might appear to be the case for hypercholesteremia and MRI parameters in the regularized logistic regression model (model D), other parameters enriched in the cSVD group such as smoking, were not returned by the algorithm (model D). In this sense, it is interesting that the random forest algorithm appeared to disregard the a priori group differences and
returned the ELISA plasma markers as important features.

The use of artificial intelligence (AI) in clinical applications has greatly increased during the past decade, and promising results have been achieved particularly in the areas of pathology, radiology and oncology (Calderaro and Kather, 2021; Kann et al., 2021; Topol, 2019). In 2018, the Food and Drug Administration (FDA) introduced a fast-track approval plan for AI and several medical applications involving machine learning have been approved by the FDA (Topol, 2019). Also in the area of cerebral disease, the benefits of machine learning are being explored, for example for the detection of aneurysms (Mensah et al., 2022). Despite the optimism that prevails in the development of AI-based clinical applications, many algorithms have yet to prove efficacy in real life clinical settings, beyond showing high predictive power in experimental applications. Closing this so-called “AI-chasm” (Topol, 2019) is essential for the full exploitation of the benefits of AI in the clinic.

In conclusion, determination of markers of vascular inflammation, platelet and neutrophil activation revealed that MPO was significantly elevated in patients with lacunar stroke, compared with mCVI and controls. Machine learning analysis indicated that particularly MPO was a predictor of cSVD and suggested a possible involvement of platelet- and neutrophil-derived inflammatory factors in the pathophysiology of cSVD. These findings might serve as a basis for future implementation of neutrophil- and platelet-based biomarkers for the characterization of cSVD and other neurodegenerative diseases.

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Declaration of Competing Interest
The authors declare no conflicts of interest.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2022.577863.

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