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# Do Concentration or Activity of Selenoproteins Change in Acute Stroke Patients? A Systematic Review and Meta-Analyses

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## Keywords

Glutathione peroxidase · Haemolysate · Plasma · Selenoprotein · Serum · Stroke

## Abstract

**Introduction:** Stroke is characterized by deleterious oxidative stress. Selenoprotein enzymes are essential endogenous antioxidants, and detailed insight into their role after stroke could define new therapeutic treatments. This systematic review aimed to elucidate how blood selenoprotein concentration and activity change in the acute phase of stroke. **Methods:** We searched PubMed, EMBASE, and Medline databases for studies measuring serial blood selenoprotein concentration or activity in acute stroke patients or in stroke patients compared to non-stroke controls. Meta-analyses of studies stratified by the type of stroke, blood

compartment, and type of selenoprotein measurement were conducted. **Results:** Eighteen studies and data from 941 stroke patients and 708 non-stroke controls were included in this review. Glutathione peroxidase (GPx) was the only identified selenoprotein, and its activity was most frequently measured. Results from 12 studies and 693 patients showed that compared to non-stroke controls in acute ischaemic stroke patients, the GPx activity increased in haemolysate (standardized mean difference [SMD]: 0.27, 95% CI: 0.07–0.47) but decreased in plasma (mean difference [MD]: –1.08 U/L, 95% CI: –1.94 to –0.22) and serum (SMD: –0.54, 95% CI: –0.91 to –0.17). From 4 identified studies in 106 acute haemorrhagic stroke patients, the GPx activity decreased in haemolysate (SMD: –0.40, 95% CI: –0.68 to –0.13)

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and remained unchanged in plasma (MD:  $-0.10$  U/L, 95% CI:  $-0.81$  to  $0.61$ ) and serum (MD:  $-5.00$  U/mL, 95% CI:  $-36.17$  to  $26.17$ ) compared to non-stroke controls. Results from studies assessing the GPx activity in the haemolysate compartment were inconsistent and characterized by high heterogeneity. **Conclusions:** Our results suggest a reduction of the blood GPx activity in acute ischaemic stroke patients, a lack of evidence regarding a role for GPx in haemorrhagic stroke patients, and insufficient evidence for other selenoproteins.

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## Introduction

The use of antioxidant therapies is a promising direction for the development of new treatments for stroke. Oxidative stress is a common feature of ischaemic and haemorrhagic stroke [1], and several preclinical studies [2–5] have demonstrated that an increase in antioxidant enzymes could be protective against stroke. Since oxidative stress is a key factor in the pathophysiology of stroke and could be used to develop novel therapies, further understanding of endogenous antioxidant mechanisms occurring after stroke is crucial.

Some selenium-dependent proteins, or selenoproteins, are key elements in the endogenous antioxidant system. One of the most important selenoprotein enzymes included in the first line of antioxidant defence is glutathione peroxidase (GPx), which functions to neutralize hydrogen peroxide ( $H_2O_2$ ) generated by the dismutation of superoxide anions [6].  $H_2O_2$  can easily cross cell membranes, leading to wide-spread oxidative stress-induced damage during stroke [7]. As an  $H_2O_2$  scavenger, GPx may be able to interrupt this detrimental oxidative stress propagation in the brain. In agreement with a potential protective role for GPx in stroke, polymorphisms causing a reduction in its transcriptional activity are associated with increased risk of ischaemic stroke and thrombosis [8–10]. Furthermore, in addition to increasing the transcription of several selenoprotein genes in the brain, intraperitoneal injection of selenium-containing peptides in a mouse model of intracerebral haemorrhage (ICH) results in the reduction of oxidative stress, excitotoxicity, and consequently haematoma volume [3]. One of the most promising antioxidant clinical trials in stroke concerned the use of ebselen, a seleno-organic compound with mimetic antioxidant activity to GPx. Oral administration of ebselen improved neurological deficits and reduced

ischaemic volume in rodent models of focal cerebral ischaemia but, in clinical trials, showed beneficial effects only until 1 month post-stroke [5, 11].

To date, the precise role of selenoproteins in the aetiology and pathophysiology of stroke is still controversial. In this study, we aimed to clarify how selenoprotein concentration and activity change in the acute phase of stroke by undertaking a systematic review and meta-analysis of observational clinical studies.

## Material and Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines, and the protocol was preregistered in the PROSPERO database (CRD42020193646).

### Search Strategy and Selection Criteria

The search of PubMed and OVID (EMBASE and Medline) was conducted up to 7th December 2020. References were also searched for relevant articles and reviews, with no additional studies found.

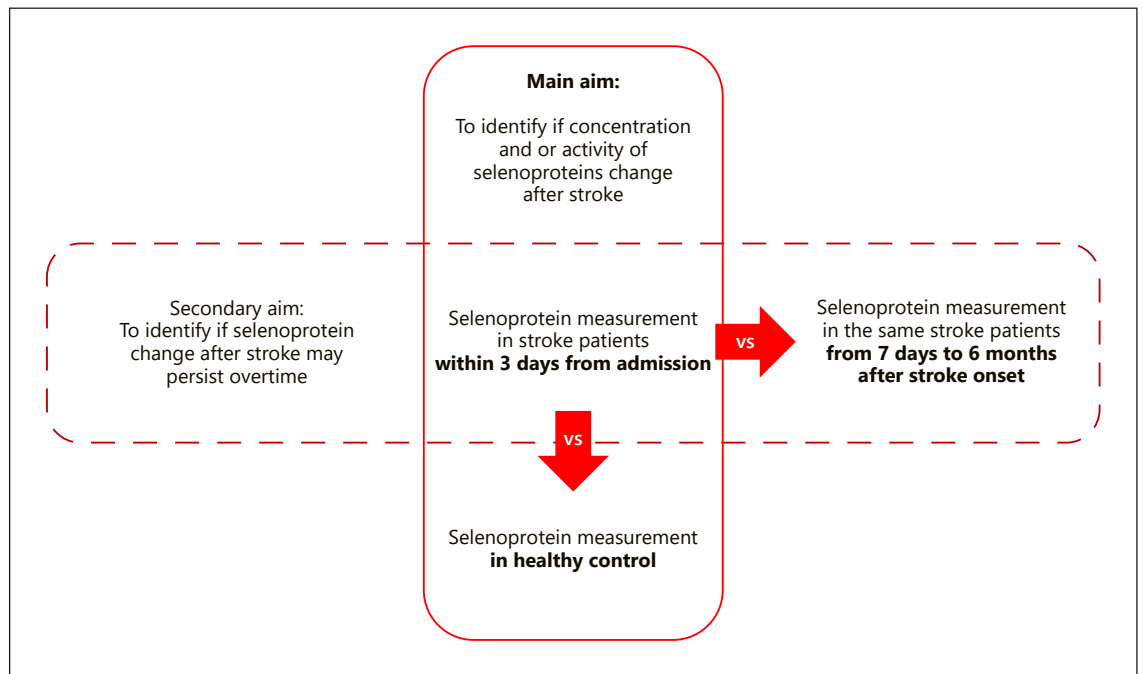
Four authors (S.T., I.-E.M., L.R., and M.E.D.H.) independently screened articles for inclusion based on title, abstract, and full text. Observational studies were eligible if they measured selenoprotein concentration and/or activity in blood compartments (haemolysate, serum, plasma, or whole blood) in people aged 18 years or over, with a diagnosis of ischaemic or haemorrhagic stroke (for keyword search strategy see online suppl. section S1; see [www.karger.com/doi/10.1159/000520856](http://www.karger.com/doi/10.1159/000520856) for all online suppl. material). Based on the aims of this study, articles were further considered based on comparison between at least one of the following: selenoprotein concentration and/or activity in stroke patients sampled within 3 days of hospital admission and non-stroke control, and selenoprotein concentration and/or activity in stroke patients sampled within 3 days of hospital admission and the same patients from 7 days to 6 months after stroke onset (Fig. 1; for detailed inclusion/exclusion criteria, see online suppl. section S2).

### Data Extraction and Quality Assessment

Three authors (S.T., I.-E.M., and L.R.) independently performed the data extraction, and all discrepancies were resolved by discussion. The quality of the articles was assessed using a modified 14-point National Heart, Lung, and Blood Institute (NIHLB) questionnaire for observational and cross-sectional studies (online suppl. data S3). Based on the number of positive answers, 4 authors (S.T., I.-E.M., L.R., and M.E.D.H.) independently and arbitrarily assigned the range boundaries of “poor,” “fair,” and “good” as a rating of quality. This range was discussed and agreed between the review team. A number of positive answers  $\leq 4$  define a “poor” rating, between 5 and 9 define a “fair” rating,  $\geq 10$  define a “good” rating.

### Statistical Analysis

All statistical analyses were performed using Review Manager version 5.3 software. The main characteristics of all included articles were collected for narrative synthesis and are shown in Table 1 and online Supplementary Table S4. For the meta-

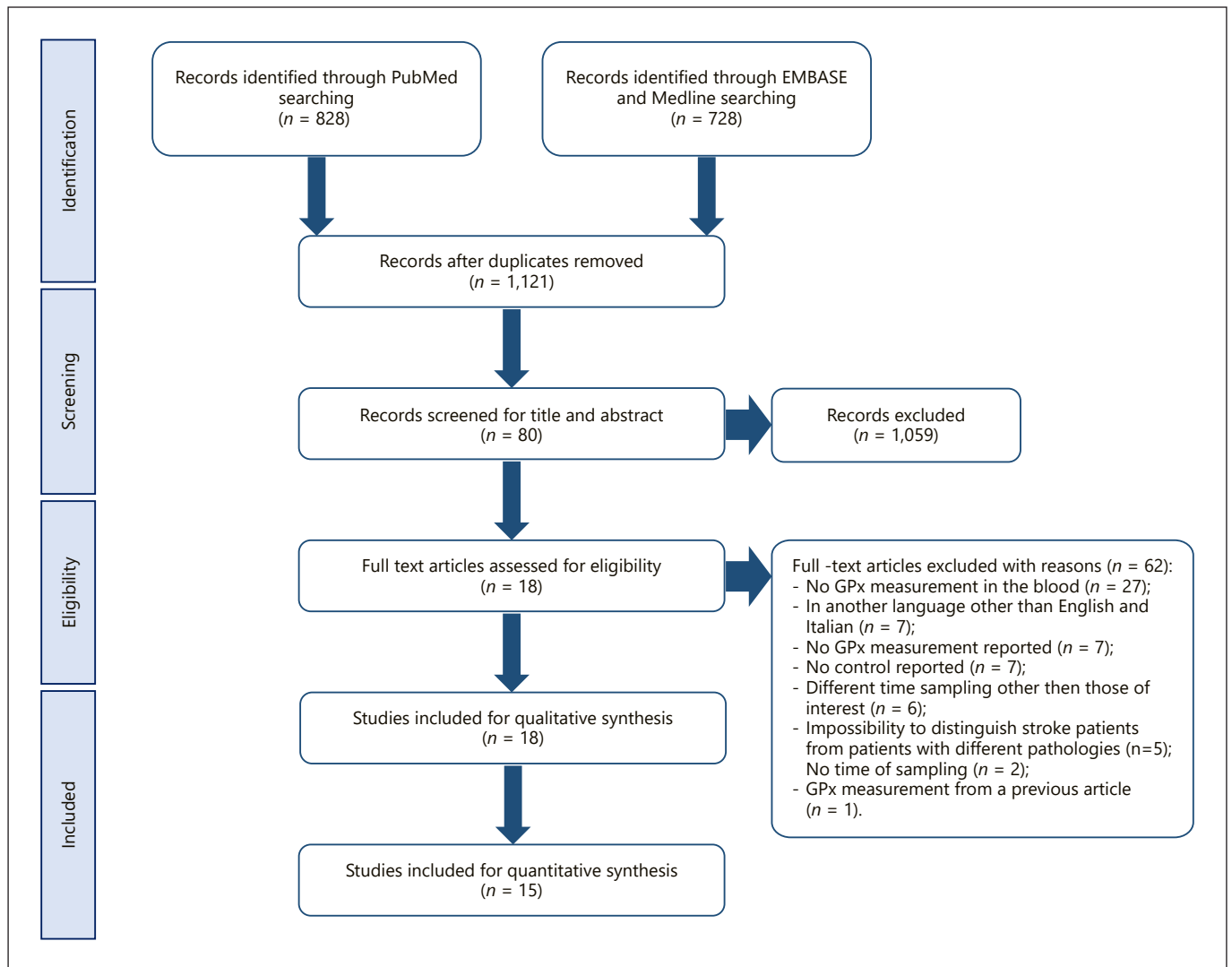


**Fig. 1.** Study scheme illustrating the 2 aims of the systematic review.

**Table 1.** Overview of the included study characteristics [12–29]

Author	Stroke type	Blood compartment	Unit of measurement	Assay	Country of study	Time of sampling, h	Sample size stroke (ischaemic/haemorrhagic)/control	Time of follow-up
Demirkaya et al. [12]	Ischaemic stroke	Haemolysate	U/gHb	Colorimetric	Turkey	<24	34/30	7 d
Ljubisavljevic et al. [13]	Ischaemic stroke	Haemolysate	U/gHb	Colorimetric	Serbia	<12	40/35	–
Ozbey et al. [14]	Ischaemic stroke	Haemolysate	U/gHb	Colorimetric	Turkey	<72	28/28	7 d
Sudha et al. [15]	Ischaemic-haemorrhagic stroke	Haemolysate	μmol NADPH oxidized/min/gHb	Colorimetric	India	<48	27/14/43	1 mo
Žitňanová et al. [16]	Ischaemic stroke	Haemolysate	U/gHb	Colorimetric	Slovakia	<24	82/81	7 d
Kolesnichenko et al. [17]	Ischaemic stroke	Haemolysate – plasma	nmol/min per 1 g of protein	Colorimetric	Russia	<72	75/23	–
Chen et al. [18]	Haemorrhagic stroke	Haemolysate	U/gHb	Colorimetric	China	<72	64/114	–
Lutsky et al. [19]	Ischaemic-haemorrhagic stroke	NR	μM/min	NR	Russia	<24	50/50/20	–
Aygul et al. [20]	Ischaemic stroke	Plasma	U/L	Colorimetric	Turkey	<24	19/20	–
Cherubini et al. [21]	Ischaemic stroke	Plasma	(μmol/L NADPH oxidized)/min/mL	Colorimetric	Italy	<72	38/37	7 d
Cojocar et al. [22]	Ischaemic stroke	Plasma	(μmol/L NADPH oxidized)/min/mL	Colorimetric	Romania	<24	57/51	7 d
Aygul et al. [23]	Haemorrhagic stroke	Plasma	U/L	Colorimetric	Turkey	<24	17/20	–
Liu et al. [24]	Ischaemic stroke	Serum	pg/mL	ELISA kit	China	<24	216/100	–
Milanlioglu et al. [25]	Ischaemic stroke	Serum	U/mL	Colorimetric	Turkey	<24	45/30	21 d
Yildirim et al. [26]	Ischaemic stroke	Serum	nmol/min/mL	Colorimetric	Turkey	<48	32/18	–
Zimmermann et al. [27]	Ischaemic stroke	Serum	U/L	Colorimetric	Germany	<12	11/17	7 d
Watanabe et al. [28]	Haemorrhagic stroke	Serum	U/mL	Colorimetric	Japan	<24	12/11	11 d
Sheikh et al. [29]	Ischaemic stroke	Whole blood	U/L	Colorimetric	Iran	<72	30/30	–

Studies sorted by the blood compartment and type of stroke.



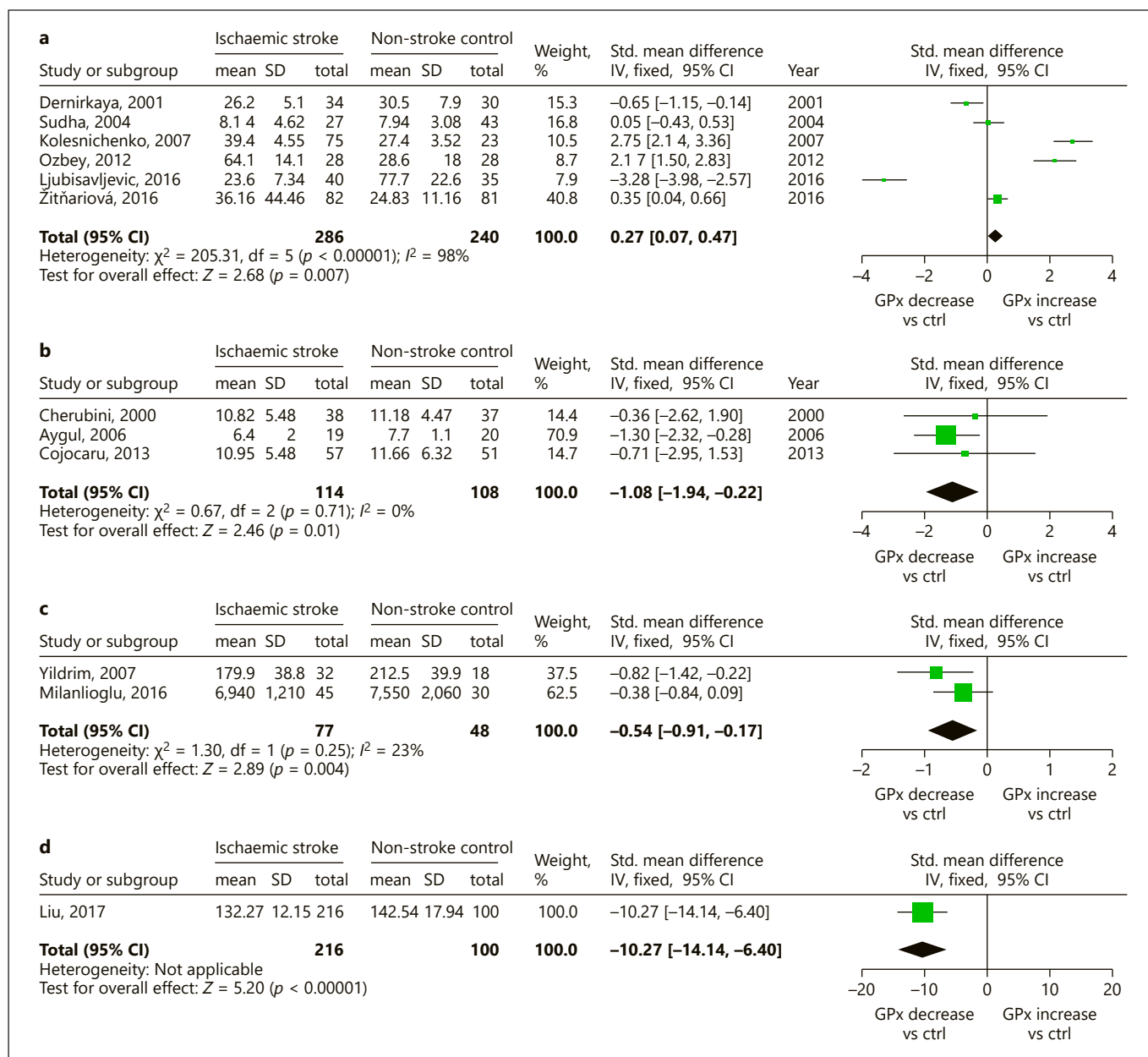
**Fig. 2.** PRISMA flowchart showing search strategy and the number of included/excluded studies for the systematic review and meta-analysis. GPx, glutathione peroxidase; PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis.

analysis, studies were synthesized considering the GPx activity or concentration measurement in the blood. Sample size per group, mean of the blood GPx activity or concentration, and their standard deviation (SD), standard error, or interquartile range were extracted. When possible, studies measuring the GPx activity or concentration in the same compartment were standardized for unit of measurement. Depending on the blood compartment, the following units of measurement were used: U/gHb for haemolysate GPx activity, U/L for plasma GPx activity, U/L for serum GPx activity, pg/mL for serum GPx concentration, and U/L for whole blood GPx activity. When raw values were shown in a graph only, the online graphical tool WebPlot-Digitizer (<https://automeris.io/WebPlotDigitizer/>) was used to extract data from figures. Where interquartile range was report-

ed, SD was measured using the method reported in the study by Wan et al. [30]. When GPx measurement was stratified in patients based on stroke severity or outcome, the groups were combined using a weighted mean.

For the secondary analysis, a crossover design was preferred. SD and standard error were estimated and used for a generic inverse-variance outcome analysis. An additional analysis was conducted investigating a possible correlation with National Institutes of Health Stroke Scale (NIHSS) severity and modified Rankin Scale (mRS) outcome. For this purpose, data from studies showing the mean GPx activity in patients stratified for the NIHSS or mRS were also extracted.

Heterogeneity among studies was assessed by Cochrane Q and  $I^2$  statistics. Based on heterogeneity and variance between studies,



**Fig. 3.** Comparison of GPx activity in haemolysate (a), plasma (b), and serum (c), and GPx concentration in serum (d) of ischaemic stroke patients and non-stroke controls. Forest plot of fixed effect of SMD (a, c) or MD (b, d) and 95% CI. GPx, glutathione peroxidase; MD, mean difference; SMD, standardized mean difference.

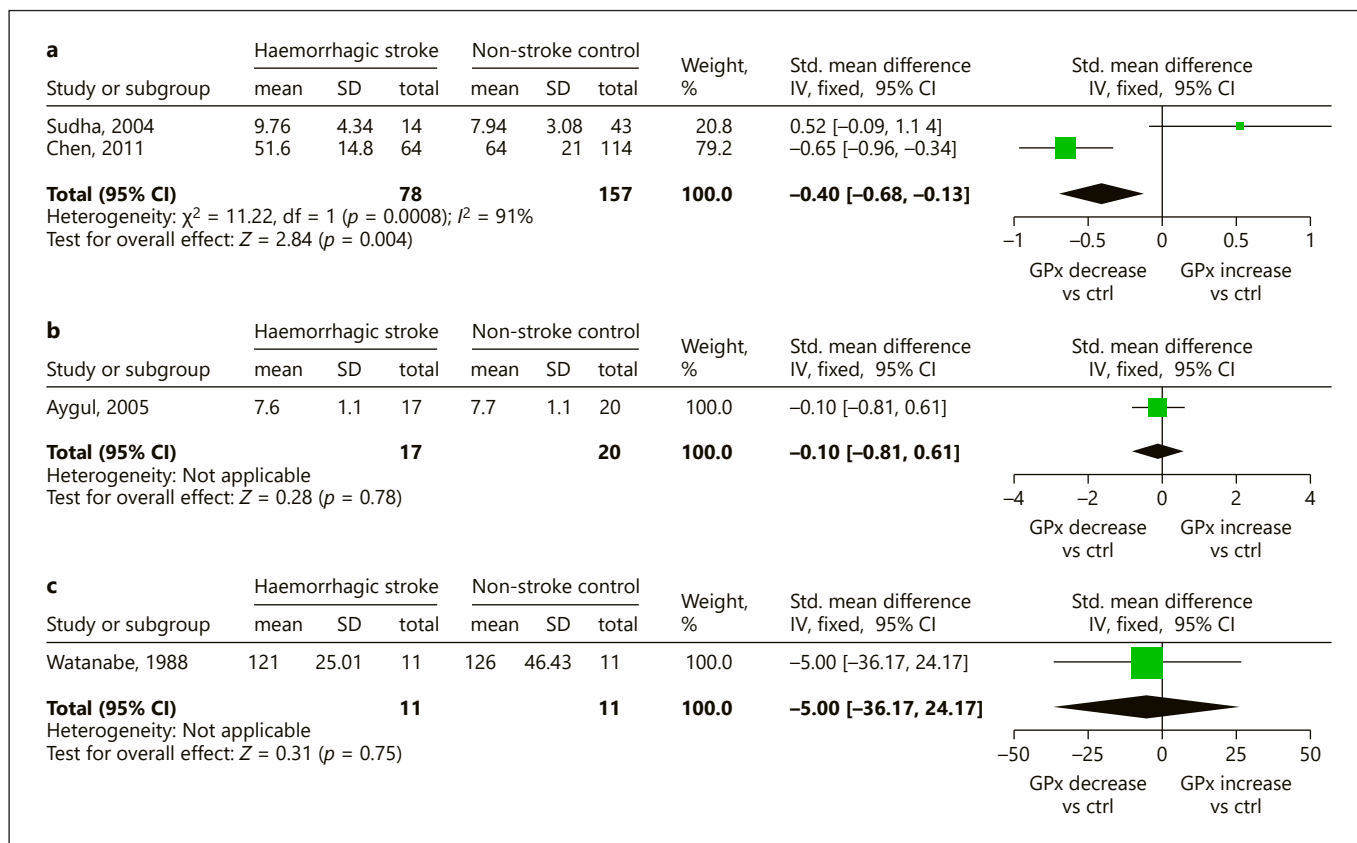
mean difference (MD) or standardized mean difference (SMD) was preferred. When SMD was applied, not normalized units of measurement were adopted. Considering the similarity of the studies in terms of design, patients recruited, and outcome measured, including time of sampling and compartment analyzed, fixed-effect meta-analysis has been preferred.

## Results

### Descriptive Analysis

The initial PubMed search identified 828 studies, and an additional search on OVID (EMBASE and Medline) identified 728 studies. After duplicate removal, 1,121 studies were identified and screened for the inclusion/ex-





**Fig. 4.** Comparison of GPx activity in haemolysate (a), plasma (b), and serum (c) of haemorrhagic stroke patients and non-stroke control. Forest plot of fixed effect of SMD (a) or MD (b, c) and 95% CI. GPx, glutathione peroxidase; MD, mean difference; SMD, standardized mean difference.

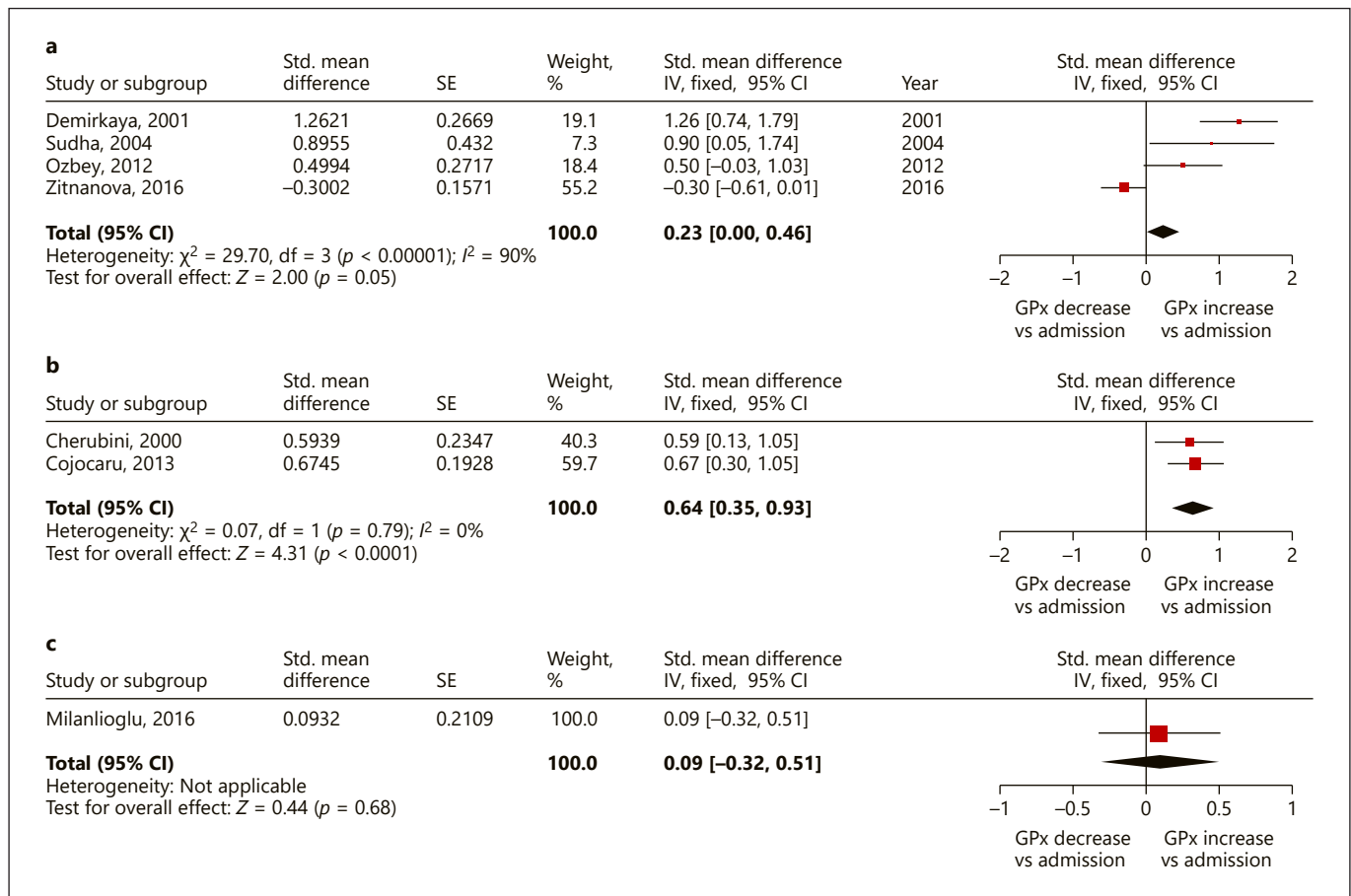
clusion criteria. All included studies measured the activity/concentration of the same selenoprotein, GPx. Eighteen studies were eligible for qualitative analysis, 16 of which were also eligible for quantitative analysis (Fig. 2).

Overall, data from 941 stroke patients were included in this review, with a mean age of ~65 years, with all studies conducted in Asia and Europe. Most of the clinical patient and control characteristics were not reported. Only a few studies reported the frequency of excess alcohol consumption ( $n = 3$ ), smoking status ( $n = 8$ ), hypertension ( $n = 9$ ), and diabetes in the stroke patients ( $n = 7$ ). Only 1 study recruited patients with a previous history of stroke, whereas in most of the studies, this information was not reported. As a possible confounding factor, we sought dietary information, but no studies reported these data, and instead, 4 studies excluded stroke patients who had been using antioxidants during the months preceding the enrolment (see online suppl. Table S4).

Most of the studies ( $n = 15$ ) investigated GPx change in ischemic stroke patients, whereas only 5 investigated GPx change in haemorrhagic stroke patients. GPx activity was the measurement most commonly analyzed ( $n = 17$ ). Only 1 study analyzed GPx concentration in serum.

In order to minimize variance and heterogeneity of the studies, they were stratified by the type of stroke, blood compartments, and GPx concentration/activity (see online suppl. section S6). Since 2 studies either did not report the blood compartment from which the analysis was performed [19] or included 7/11 patients with minor or no permanent deficit in the ischaemic stroke group [27], they were only eligible for the qualitative analysis.

The quality rate of most included articles was fair (67%), and the most frequent value was 6 (5–7 points; online suppl. Table S5). Lower scores were mostly due to the lack of information regarding the participation rate of eligible persons, sample size justification, if outcome assessors were blinded to the exposure status of participants, and if con-



**Fig. 5.** Comparison of GPx activity in the haemolysate (a), plasma (b), and serum (c) of ischaemic stroke patients between 7 days and 1 month from stroke onset and the same patients at admission. Forest plots of fixed effect of SMD and 95% CI. GPx, glutathione peroxidase; SMD, standardized mean difference.

founding variables were measured and adjusted statistically for their impact. Given the small number of studies, it was not possible to express publication bias as a funnel plot.

### Quantitative Synthesis: Meta-Analyses

#### Main Objective

In haemolysate, the GPx activity was increased in ischaemic stroke patients compared to non-stroke controls (SMD: 0.27, 95% CI: 0.07–0.47, Fig. 3a). In contrast, the GPx activity was decreased in haemorrhagic stroke patients compared to non-stroke controls (SMD: -0.40, 95% CI: -0.68 to -0.13, Fig. 4a). In both analyses, despite the studies being grouped to minimize the interstudy heterogeneity,  $I^2$  was found to be  $>90\%$ , indicating substantial heterogeneity. This suggests a serious inconsistency between the studies, with SMD ranging between -3.28 and 2.75 in ischaemic stroke analysis, and -0.65 and 0.52

in haemorrhagic stroke analysis. To understand whether study quality could affect the heterogeneity, analysis of studies grouped for the quality rate has been performed. Two ischaemic stroke studies and 1 haemorrhagic stroke study were rated as “poor” and showed a higher effect size than the studies rated as “good/fair” (see online suppl. data S7 and S8). These data might suggest a relationship between study quality and effect size.

In plasma, the GPx activity was significantly reduced in ischaemic stroke patients compared to non-stroke controls (MD: -1.08 U/L, 95% CI: -1.94 to -0.22, Fig. 3b). One study [17] used different units of measurements to the others, and the post hoc decision was made to exclude it from the meta-analysis. In the same compartment, the GPx activity remained unchanged in haemorrhagic stroke patients compared to non-stroke controls (MD: -0.10 U/L, 95% CI: -0.81 to 0.61, Fig. 4b).



In serum, the GPx activity and concentration were significantly reduced in ischaemic stroke patients compared to non-stroke controls (SMD:  $-0.54$ , 95% CI:  $-0.91$  to  $-0.17$ ; MD:  $-10.27$  pg/mL, 95% CI:  $-14.14$  to  $-6.40$ , respectively, Fig. 3c, d). In the same compartment, the GPx activity remained unchanged in haemorrhagic stroke patients compared to non-stroke controls (MD:  $-5.00$  U/mL, 95% CI:  $-36.17$  to  $26.17$ , Fig. 4c).

The whole blood compartment was investigated in only 1 study, which reported a comparison between ischaemic stroke patients and non-stroke controls. From this, the GPx activity was significantly reduced in ischemic stroke patients compared to non-stroke controls (SMD:  $-0.58$ , 95% CI:  $-1.10$  to  $-0.06$ , online suppl. Fig. S9).

According to the results from plasma, serum, and whole blood, the study by Lutsky et al. [19], which did not report in which blood compartment the analyses were performed, showed a significant decrease of the GPx activity in ischaemic stroke patients compared to non-stroke controls (SMD:  $-4.81$ , 95% CI:  $-5.77$  to  $-3.84$ , online suppl. Fig. S10a). However, the haemorrhagic stroke analysis in this study was not consistent with the other 4 included studies. Whereas a possible decrease of the GPx activity in haemolysate and no changes in plasma and serum are reported (Fig. 4), Lutsky et al. [19] described a significant increase of the GPx activity in haemorrhagic stroke patients compared to non-stroke controls (SMD:  $39.88$ , 95% CI:  $33.06$ – $46.70$ , online suppl. Fig. S10b).

### Secondary Objective

For the secondary objective, 8 studies included follow-up analysis: 7 measured the activity of GPx in ischaemic stroke patients, whereas only 1 study included haemorrhagic stroke patients. In haemolysate of ischaemic stroke patients, the GPx activity increased between 7 days and 1 month from stroke onset, compared to the value of the same patients on admission (SMD:  $0.23$ , 95% CI:  $0.00$ – $0.46$ , Fig. 5a). Although subgroup analysis of the study quality rate showed that the poor quality rated study had a higher effect size, this did not deeply affect the result in the haemolysate (see online suppl. data S11).

Only 2 follow-up ischaemic stroke studies that investigated the GPx activity in plasma were found, reporting an increase after 7 days from ischaemic stroke onset compared to the value in the same patients at admission (SMD:  $0.64$ , 95% CI:  $0.35$ – $0.93$ , Fig. 5b). Two studies showing, respectively, the follow-up from ischemic and haemorrhagic stroke patients that evaluated serum were identified. The GPx activity was not significantly different

in ischaemic stroke patients after 21 days from stroke onset compared to the same patients at admission (SMD:  $0.09$ , 95% CI:  $-0.32$  to  $0.51$ , Fig. 5c). In contrast, the only study analyzing the GPx activity in haemorrhagic stroke patients' serum showed a significant increase of the GPx activity in haemorrhagic stroke patients after 10–11 days from stroke onset compared to the same patients at admission (SMD:  $9.46$ , 95% CI:  $6.28$ – $12.69$ , online suppl. Fig. S12).

### GPx Activity Compared to Stroke Severity and Outcome

For ischaemic stroke studies, an additional analysis was performed through comparison between measurements of GPx activity in patients grouped by the NIHSS and mRS outcome. Only 2 studies sub-divided the stroke patients by the NIHSS. From these, in both haemolysate and plasma compartments, the GPx activity in patients with a higher stroke severity (NIHSS  $> 10$ ) was increased compared to mild or moderate stroke severity patients (NIHSS  $\leq 10$ ; SMD:  $0.67$ , 95% CI:  $0.25$ – $1.10$ ; MD:  $4.57$  U/g protein, 95% CI:  $3.96$ – $5.18$ ; online suppl. Fig. S13).

Furthermore, 2 studies analyzed the GPx activity in the haemolysate through sub-grouping the stroke patients into severe (mRS  $> 3$ ) and mild (mRS  $\leq 3$ ) outcomes. A meta-analysis from these 2 studies showed that a decrease in the GPx activity in the haemolysate may be related to worse outcome in ischaemic stroke patients (MD:  $-4.62$  U/gHb, 95% CI:  $-7.77$  to  $-1.47$ , online suppl. Fig. S14).

## Discussion

In this systematic review, we aimed to investigate whether selenoprotein concentrations or activity changes in acute ischaemic and haemorrhagic stroke patients. Despite the use of 17 different entry terms to identify all the records containing selenoprotein measurement, only studies showing GPx change after stroke were identified. Overall, we found that the GPx activity was not consistent between the 2 types of stroke. However, since only a few studies were identified investigating the role of GPx after haemorrhagic stroke, a comparison between GPx activity in ischaemic and haemorrhagic stroke patients was not possible.

In haemolysate, from both ischaemic and haemorrhagic stroke patients assessed within 3 days of stroke onset and compared to non-stroke controls, the hetero-

geneity of the activity was >90%. This high heterogeneity, mostly due to contradictory results between the studies, may be explained by significant differences of patient and control characteristics in terms of confounding factors. In ischaemic stroke patients, the haemolysate GPx activity was found to decrease, increase, or stay unchanged compared to non-stroke controls. Of those studies, 2 recruited first-ever stroke patients and only one provided information about previous history of stroke (online suppl. Table S4). Likewise, conflicting results of haemolysate GPx activity are shown in haemorrhagic stroke patients compared to non-stroke controls. Although most of the patients' medical history or their lifestyle information was not reported, it is possible that previous stroke history may in part condition the amount of GPx in erythrocytes in further strokes, and this could potentially explain the large heterogeneity in reported findings. Due to the absence of long-term follow-up studies or studies showing patients sub-grouped by their previous stroke history, the latter hypothesis cannot be assessed in this review.

In contrast to haemolysate, in plasma, serum, and whole blood of ischaemic stroke patients assessed within 3 days of stroke onset, GPx activity and concentration decreased compared to non-stroke controls. Although only 7 studies investigated this aspect, the consistency of their results and the absence of heterogeneity suggest a high reliability. However, these results are not comparable to what the excluded study [31] reported, which was a significant increase of GPx activity in ischaemic stroke patients within 7 days of stroke onset compared to non-stroke controls. The reduction of GPx activity in plasma, serum, and whole blood may be caused by a decrease in the concentration of GPx3 isoform in particular, which is the major antioxidant enzyme in plasma. Accordingly, Akhter et al. [32] showed a significant decrease in GPx3 concentration in ischaemic stroke patients during the early acute phase.

Compared to patients with ischaemic stroke, in both blood compartments, the GPx activity from ICH and subarachnoid haemorrhage patients was unchanged compared to non-stroke controls. However, the only 2 identified studies, which included <20 patients each, are in conflict with a recently published study (not included in this review because it was published after the search was performed), showing that in patients with subarachnoid haemorrhagic stroke, there was a 133% increase of the plasma GPx activity compared to non-stroke controls [33].

The follow-up meta-analyses included 8 studies. Most of them ( $n = 7$ ) concerned the follow-up of ischaemic stroke patients, whereas only 1 investigated the follow-up of haemorrhagic stroke patients. In the haemolysate of ischaemic stroke patients, the GPx activity remains elevated 7 days after stroke onset. However, the heterogeneity of the activity in this analysis was high. This can be in part attributable to the anomalous result from 1 study which used a different approach to prepare the haemolysate and biochemically assess GPx activity. However, this would not explain such high inconsistency of results of this study compared to the others.

In plasma and serum of follow-up ischaemic stroke patients, the GPx activity reached the non-stroke control values, and these results are in line with what Akhter et al. [32] reported on GPx3. Regarding haemorrhagic stroke, only 1 follow-up study on subarachnoid haemorrhage was found. This showed an approximately 70 fold increase of the GPx activity in serum of follow-up patients compared to the same patients at admission and to non-stroke controls. However, considering the low sample size from this study and a more recent opposing result from Jarocka-Karpowicz et al. [33], showing a decrease of plasma GPx activity in the follow-up of subarachnoid haemorrhagic stroke patients compared to the same patients within 24 h of admission, further investigation should be conducted.

Analyses of stroke severity suggest that an increase in haemolysate and plasma GPx activity might correlate with higher NIHSS. However, these 2 meta-analyses either resulted in high heterogeneity or included only 1 study. Furthermore, these results differ from the excluded study by Alexandrova et al. [31], where GPx activity in whole blood did not reflect the severity of neurological deficit measured with the Mathew scale.

Regarding haemorrhagic stroke, no studies were found comparing GPx activity or concentration in the blood in relation to the neurological scale. However, a recent study from Jarocka-Karpowicz et al. [33], assessed plasma GPx concentration relating to the World Federation of Neurological Surgeons Scale (WFNS). Although no differences were found in patients with worse neurological condition, this study showed a non-significant trend in increase in plasma GPx concentration in patients with brain edema compared to those with no edema [33].

Finally, a meta-analysis comparing the GPx activity of ischaemic stroke patients and mRS outcome was performed. The only 2 studies found showed a significant decrease of the haemolysate GPx activity in patients with worse outcome. A decrease in the GPx activity of eryth-

rocytes may cause lipid peroxidation of their cellular membranes with consequent erythrocyte haemolysis [15], which may result in a worse outcome after stroke. However, further analyses are necessary to confirm these data.

Study quality of the articles was assessed using a modified 14-point NIHLB questionnaire, finding a mean score of 6 and generally fair quality. Yet, poor information of the eligibility rate, justification of sample size, blind assessment, and confounding variables were identified. Furthermore, the small number of studies and their small sample size did not allow for the creation of a reliable funnel plot to assess publication bias, and, since 7 studies were excluded because they were written in a different language (Russian and Spanish) other than English and Italian, this may have led to some bias. Sub-group analyses (see online suppl. data S7, S8, S11) by study quality (poor vs. fair/good) suggested a relationship between study quality and effect size, with the poor quality studies tending to report more extreme effect sizes.

#### *Limitations*

This systematic review has some limitations, which are mainly related to the characteristics of the studies included. Despite efforts to minimize the variance between the included studies through standardization of the units of measurement, some of them showed large differences in terms of result direction and magnitude compared to the other studies. This might in part be due to technical variation in the methodology to assess the GPx activity. Heterogeneity may also be to do with bias introduced by the design of these observational studies; 4 of the studies were rated as “poor quality” using the modified 14-point NIHLB questionnaire. Sub-group analyses revealed that effect size might be related to study quality.

The high variance found, especially in the haemolysate compartment, make the results from these analyses difficult to draw conclusions from. Another important limitation is represented by the restriction of this systematic review to the blood compartment. Selenoprotein changes in the central nervous system, where oxidative stress-induced damage is more considerable, were not addressed. To the best of our knowledge, there is only 1 study addressing selenoprotein measurement in autopsied brains of patients with or without cerebral infarction. Tazikawa et al. [34] described the cellular origin of GPx in the brain, showing that, whereas in the control tissue GPx staining was limited to neurons and glial cells, in ischaemic stroke patients, GPx staining was also found in the cytosol of macrophages within the core lesion.

However, no studies were found investigating GPx concentration or activity in the brain. In contrast, several studies with contradictory results analyzed how GPx activity changed in the cerebrospinal fluid (CSF) of haemorrhagic stroke patients. Some of them showed no significant differences between patients with ruptured or unruptured aneurism [35] and patients with or without ICH [36]. However, Pyne-Geithman et al. showed a significant increase of the GPx activity in the CSF of subarachnoid haemorrhagic stroke patients, which was raised in the presence of vasospasm [37]. Accordingly, Papacocca et al. [38] also found significantly increased GPx in the CSF of ICH stroke patients compared to non-stroke controls. It is evident that the behaviour of GPx may be specific to the stroke type. However, further studies are required to better understand the role of GPx in the pathophysiology of stroke.

#### **Conclusion**

This systematic review shows that there is a distinct lack of evidence to define all the selenoprotein trends after stroke. However, GPx analysis suggests a reduction of GPx activity in plasma, serum, and whole blood of ischaemic stroke patients assessed within 3 days of stroke onset compared to non-stroke controls. This may be due to a decrease in GPx3 concentration in these compartments, with this being the major isoform transported in the blood. In addition, this review suggests that a decrease in the erythrocyte GPx activity might correlate with worse outcome in ischaemic stroke patients. The inconsistency of the further results from this review and the small number of studies meeting the inclusion criteria make it impossible to draw any other conclusion. Therefore, further high-quality studies are required to provide more insights on how selenoprotein concentration and activity change after stroke and to identify whether selenium supplementation may induce a favourable effect on patients with stroke. Future observational studies should (i) consider higher sample sizes for adequate statistical power; (ii) report more fully details about patients' medical history and consider possible confounding factors, such as dietary vitamin and mineral intake of patients and controls; (iii) use a clearly stated reproducible and universal method to measure selenoprotein concentration and activity; and (iv) use a longitudinal cohort study, where the stroke population is followed over time.

## Statement of Ethics

An ethics statement was not required for this study type; no human or animal subjects or materials were used.

## Conflict of Interest Statement

The authors declare no conflicts of interest.

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## Author Contributions

S.T. and T.N.S. conceived the study and S.T. designed it. S.T., I.-E.M., L.R., and M.E.D.H. performed the systematic review, with input from S.M.A., C.J.S., T.N.S., and S.R. S.T. performed the statistical analysis assisted by S.R. S.M.A., C.J.S., T.N.S., S.R., and J.R.G. provided input to the study design, data interpretation, and commented on draft manuscript.

## Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further enquiries can be directed to the corresponding author.

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