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Blood glutathione as a surrogate marker of cancer tissue glutathione S-transferase activity in non-small cell lung cancer and squamous cell carcinoma of the head and neck

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Abstract

The identification of markers predicting the response to therapy is of the utmost importance in oncology. Several authors have suggested that increased levels of glutathione (GSH) and glutathione S-transferase (GST) activity might be meaningful predictors of poor responsiveness to chemotherapy in several human cancers, but the biological assays have not been standardised and published studies show conflicting evidence. The aim of the present study was to select a validated panel of tests to assess the GST/GSH system in a clinical setting. Matched blood and tissue samples (normal and malignant) from 52 cancer patients with either non-small cell lung cancer (NSCLC) or head and neck squamous cell carcinoma (SCCHN) were investigated. GSH levels and GST activity were higher in cancer tissues than in matched normal tissues in both malignancies. The difference was statistically significant in NSCLC ($P=0.0004$ and $P=0.0002$, for GSH and GST, respectively) and borderline in SCCHN ($P=0.03$ and $P=0.02$, for GSH and GST, respectively). Moreover a strong correlation was found between the GSH level in whole blood and GST activity in cancer tissue in both malignancies ($P=0.003$, $r=0.53$ in NSCLC, $P<0.0001$, $r=0.89$ in SCCHN). In conclusion, reliable and robust methods for routine use in tissue extracts and in whole blood have been validated. Our finding regarding the GSH level in blood indicates that circulating GSH could have a clinical relevance as a surrogate marker of GST activity in tumour tissue.

Keywords: Glutathione; Glutathione S-transferase; Non-small cell lung cancer (NSCLC); Head and neck squamous cell carcinoma (SCCHN); Surrogate markers; Resistance to chemotherapy

1. Introduction

Resistance to chemotherapy is one of the most important clinical problems in the treatment of cancer. It may either be present before treatment as intrinsic resistance [1] or it can be acquired during chemotherapy [2]. The identification of resistance mechanisms and the definition of reliable predictive indicators of response to treatment in individual patients are necessary to achieve a more effective treatment of cancer. One of the most important mechanisms is the action of glutathione (GSH) and GSH-dependent detoxifying enzymes [3].

Glutathione S-transferases (GSTs) represent the most abundant and ubiquitous family of enzymes active in the cellular detoxification process [4]. GSTs are an integral part of the biphasic oxidation/conjugation system that metabolises many cytotoxic agents and other foreign compounds, including anticancer drugs [5]. Seven cytosolic GST gene sub-families (alpha, pi, sigma, kappa, theta and zeta) and several membrane bound GST isoenzymes have been identified in mammalian species [6]. These enzymes catalyse the direct coupling of reduced glutathione (GSH, γ-Glu-Cys-Gly) to electrophilic compounds (chemicals, reactive oxygen species, drugs and other xenobiotic agents).

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The GSH-conjugated compounds are usually inactive and are excreted from the organism into urine or bile [7]. There is some evidence that GST expression and GSH levels may be correlated to the outcome of chemotherapy [8].

GSH is elevated in cultured cell lines, experimental tumours and cells derived from human tumours that are resistant to anticancer drugs, particularly to alkylating agents and platinum compounds [9]. High intrinsic drug resistance of human cancer cells has been associated with increased GSH content and altered expression patterns of GST [4,9]. Cells made resistant to a variety of drugs, including doxorubicin, alkylating agents and platinum, overexpress GST activity [9]. In general, the π isoenzyme is most commonly increased in doxorubicin- and platinum-resistant tumour cells, while the α and μ isoenzymes are frequently increased in association with exposure to alkylating agents [9].

In head or neck or squamous cell carcinoma (SCCHN), circulating GSH and GST levels were found to be higher in patients with stage IV disease than in those with earlier stages [10]. In another study, a global GST score was assigned to tumour biopsies and correlated to the response to platinum-based chemotherapy: 14 of 14 patients with a low GST score responded to neoadjuvant chemotherapy, while only 4 of 9 patients with a high GST score responded in the same setting. Patients receiving treatment for relapsed disease showed the same correlation, with 7 of 10 patients with a low GST score responding, while only 2 of 23 patients in the high GST score group responded [11].

In non-small cell lung cancer (NSCLC), the response rate to cisplatin-based chemotherapy was much lower in patients with positive immunochemistry for GST-Pi in their tumour biopsies than in patients with GST-Pi-negative biopsies (3/25 versus 9/13, respectively) [12]. Finally, in another study, patients relapsing after platinum-based adjuvant chemotherapy had high levels of GST-Pi [13].

GST distribution in cancer patients has been extensively investigated, but conflicting data have been reported in the literature, probably reflecting the complexity of the GST/GSH system in different tumours, different biological samples and different analytical methodologies.

A number of studies have demonstrated an enhanced expression of the various GST isoforms in cancer tissue in comparison to normal tissue. Tsuchida and colleagues [14], using an enzyme-linked immunosorbent assay (ELISA) (in-house method), found that the GST-Pi content in cancer colon mucosa was 6-fold higher than the level found in normal mucosa. Howie and colleagues [15] showed that the concentration of GSTs, measured by a radioimmunoassay, was significantly greater in the lavage fluid taken from the area of lung associated with the tumour when compared with the normal area of tissue. Moreover, in 19 lung, 27 colon and 9 stomach cancer patients, GST-Pi concentration, detected by chromatography, was found to be significantly higher in tumour tissue then in matched normal tissue samples [16].

However, using an ELISA (in-house method), no correlation between the GST level and the presence of the malignancy was found in head and neck [17] and in bladder cancer patients [18].

In contrast, Buser and colleagues [19], using an enzymatic assay, found that high GST and GSH levels were associated with favourable clinical characteristics and good prognosis in 89 breast cancer patients.

We analysed GST activity and GSH levels in matched tissue and blood samples from NSCLC and SCCHN patients.

The primary objective of the study was to validate robust and reliable assay methods for GSH quantitation and GST activity suitable for routine clinical use by evaluating their sensitivity (analytical and functional), accuracy and precision. The existence of a correlation between blood and tissue findings was also explored.

2. Materials and methods

2.1. Patients

29 NSCLC and 23 SCCHN newly diagnosed, consecutive patients were enrolled from June 2000 to May 2001 at the European Institute of Oncology in Milan (Italy) at the time of primary surgery. Informed consent was obtained from each patient. Patients' demographic and disease characteristics are summarised in Table 1.

2.2. Biological material

2.2.1. Tissue

A sample (>200 mg) of tumour tissue (adjacent to the sample submitted for histological examination) and a sample of normal tissue were collected at surgery from each patient, immediately frozen in liquid nitrogen and stored at −80 °C until processed.

The cytosol was prepared using a modification of the conventional cytosol preparation protocol as previously described in Ref. [20]. Briefly, the tissue was pulverised in its frozen state, homogenised with 6 volumes of 50 mM phosphate buffer pH = 7.4 and centrifuged at 10 000*g* for 20 min at 4 °C. The supernatant (cytosol) was collected and stored at −80 °C until assayed. The protein concentration was determined as reported by Bradford [21] using bovine serum albumin (BSA) as a standard.

2.2.2. Blood

Approximately 8 ml of blood were collected from each patient by venipuncture before surgery: 3 ml were

Table 1
Main characteristics of patient series

		SCCHN	NSCLC
n		23	29
Age (years)		56 (29–72)	67 (28–80)
Gender (*n*)	Male	16	24
Tumour		23 SCCHN	
	Female	7	5
Stage (*n*)	Stage 1	3	10
	Stage 2	3	5
	Stage 3	3	13
	Stage 4	11	0
	Missing	3	1
Histology		23 SCC	17 adenocarcinoma; 6 SCC; 6 others
Site involved	10 tongue; 4 oropharynx; 1 mouth; 7 larynx; 1 uvula		7 RUL; 5 LLL; 3 RLL; 3 RML; 4 LL; 6 LUL; 1 RL

RUL, right upper lobe; LLL, left lower lobe; RML, right middle lobe; LL, left lobe; RLL, right lower lobe; LUL, left upper lobe; RL, right lobe; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; HN, head and neck.

immediately stored as whole blood at −80 °C until assayed; 4.5 ml were dispensed in a CTAD (sodium citrate, theophylline, adenosine, dipyridamole) tube (Beckton-Dickinson, Plymouth, UK), mixed by gentle inversion and placed immediately in an ice bath. CTAD tubes were centrifuged at 1500*g* for 20 min at 4 °C within 1 h of the collection. One-third of the supernatant plasma from the centre portion of the liquid phase was transferred into 1-ml aliquots in Eppendorf tubes and stored at −80 °C until assayed.

2.3. GSH assay

GSH levels in cytosol and whole blood were measured by a commercially available assay kit (Cayman, Ann Arbor, MI, USA). This assay kit utilises an enzymatic recycling method based on the reaction between GSH and 5.5′-dithio*bis*-2-nitrobenzoic acid (DTNB) that produces a yellow coloured compound 5-thio-2-nitrobenzoic acid (TNB). The rate of TNB production is directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 405 nm provides an accurate estimation of GSH in the sample. Due to the presence of glutathione reductase, which reduces GSSG (the disulphide dimer of glutathione) to GSH, in the reaction buffer, both GSH and GSSG are measured and the assay reflects total GSH present in the sample [22–24].

Before assaying, samples were deproteinised with 10% metaphosphoric acid (MPA) to avoid interference due to sulphhydryl groups on the proteins in the assay. 50 μl of the deproteinated sample (whole or diluted 1:3 with kit Wash Buffer) were assayed in duplicate according to the manufacturer's instructions. The GSH concentration was measured by comparison with a standard curve obtained by plotting the absorbance (405 nm) at 25 min versus GSH concentration (nmol/ml). Cytosol GSH levels were normalised for protein content (nmol/mg).

GSH levels were not assayed in plasma samples because of low levels of GSH in this matrix (data not shown).

2.4. GST activity assay

10 μl of cytosol or 50 μl of plasma were analysed by a commercially available assay kit (Novagen, Darmstadt, Germany) according to manufacturer's instructions. This kit is designed to perform a colorimetric-enzymatic assay of GST. A sample is combined with the 1-chloro-2,4-dinitrobenzene (CDNB) substrate in the supplied reaction buffer and the absorbance of the reaction is monitored at 340 nm. As most GSTs exhibit an activity towards CDNB, all of the enzyme isoforms are measured by this assay. The rate of change in A_{340} is proportional to the amount of GST activity in the sample [25].

The absorbance at 340 nm was monitored every 30 s over a period of 5 min.

GST activity of all the samples was compared with a standard (cytosol of human placenta) and was expressed as arbitrary U per mg proteins (U/mg).

2.5. Analytical evaluation

Analytical sensitivity was evaluated as the mean ± 3 standard deviation (S.D.) of eight replicates of the blank standard.

Functional sensitivity was defined as the lowest concentration of the analyte measured with an inter-assay coefficient of variation (CV) of $\leqslant 10\%$. It was obtained directly from curve fitting of the mean concentration of every sample and its respective CV over the eight measurements after a quadratic regression fit method.

Accuracy was evaluated by performing a dilution test of a cytosol sample considering the difference between the expected and observed values (percentage of recovery).

Precision was evaluated by analysing, for five consecutive runs, a duplicate of whole blood and cytosol samples; for GST activity, it was evaluated on four replicates of two cytosol samples with two different activity levels.

2.6. Statistical analysis

All statistical analyses were carried out using S-PLUS 2000 computer software, version 2. Spearman's rank order test was applied to validate significant correlations. The statistical significance of differences between normal and cancer tissue levels was evaluated using the 'paired' Wilcoxon's rank sum test. Due to the limited number of cases for each stage for both malignancies and the short time of observation, clinical correlation or survival analysis were not performed.

P values <0.05 were considered to be statistically significant.

3. Results

3.1. Analytical evaluation

Analytical sensitivity was 0.33 nmol/ml and 0.0055 U/mg for GSH quantity and GST activity, respectively.

In the *functional sensitivity* evaluation, the minimum concentration with a CV less than 10% was 0.4 nmol/ml for GSH and 0.008 U/mg of activity for GST.

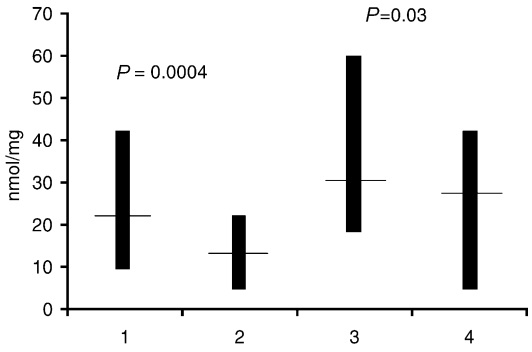

Fig. 1. Comparison of the glutathione (GSH) level between tumour and normal tissue in non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (SCCHN) cancers. 1 = lung tumour tissue; 2 = lung normal tissue; 3 = head and neck tumour tissue; 4 = head and neck normal tissue; horizontal lines represent the medians; the vertical bars represent the 10th and 90th percentile range.

In the *accuracy* evaluation, recovery was between 99 and 103% for GSH and between 112 and 133% for GST activity.

In the statistical analysis of the *precision* studies, the inter- and the intra-assay CVs were below 12 and 5% of variability for GSH; for GST activity inter- and intra-assay CV were under 9% of variability in the high activity level sample and under 14% of variability in the low activity level sample.

3.2. GSH

GSH levels were measured in whole blood and in cytosol samples of both tumour and normal tissue samples from 29/29 patients with NSCLC and 23/23 with SCCHN. Results are summarised in Table 2. Mean

Table 2
Summary of the GSH levels results

Variable	Cancer tissue (nmol/mg)	Normal tissue (nmol/mg)	Whole blood (nmol/ml)
Overall			
Mean	28.5	18.6	477
S.D.	15.3	10.7	116
Median	26.0	16.3	458
10–90th percentile	11.7–48.1	6.0–38.8	350–620
n	48	48	52
NSCLC			
Mean	24.1	13.6	516
S.D.	12.0	6.5	117
Median	22.1	13.7	494
10–90th percentile	11.2–42.0	6.0–20.9	383–681
n	28	28	29
SCCHN			
Mean	34.8	25.7	428
S.D.	17.4	13.0	97
Median	30.5	27.9	426
10–90th percentile	18.7–59.9	8.2–40.9	317–566
n	20	20	23

GSH, glutathione; S.D., standard deviation.

GSH level in whole blood was 516 nmol/ml (S.D. = 117 nmol/ml) in NSCLC and 428 nmol/ml (S.D. = 97 nmol/ml) in SCCHN, respectively. In NSCLC, the GSH level in the cytosol was 24.1 nmol/mg (S.D. = 12.0 nmol/mg) in the cancer tissue and 13.6 nmol/mg (S.D. = 6.5 nmol/mg) in the matched normal tissue samples. In SCCHN, GSH level was 34.8 nmol/mg (S.D. = 17.4 nmol/mg) in the cancer tissue and 25.7 nmol/mg (S.D. = 13.0 nmol/mg) in the corresponding normal tissue. GSH level was significantly higher in the cancer than in normal tissue in both tumour types ($P = 0.0004$ for NSCLC, $P = 0.03$ for SCCHN, Fig. 1). GSH levels in the cancer tissue were significantly correlated with whole blood GSH levels in the NSCLC ($P = 0.006$; $r = 0.5$, Fig. 2a), but not in the SCCHN ($P = 0.11$; $r = 0.35$, Fig. 2b). On the contrary, GSH levels in normal tissue and blood were not correlated in both cancer types (data not shown).

3.3. GST activity

Total GST activity was measured in the plasma and cytosol.

We tested plasma samples from 21/29 NSCLC patients and 15/23 SCCHN patients. The level of GST activity was so close to the sensitivity threshold of the

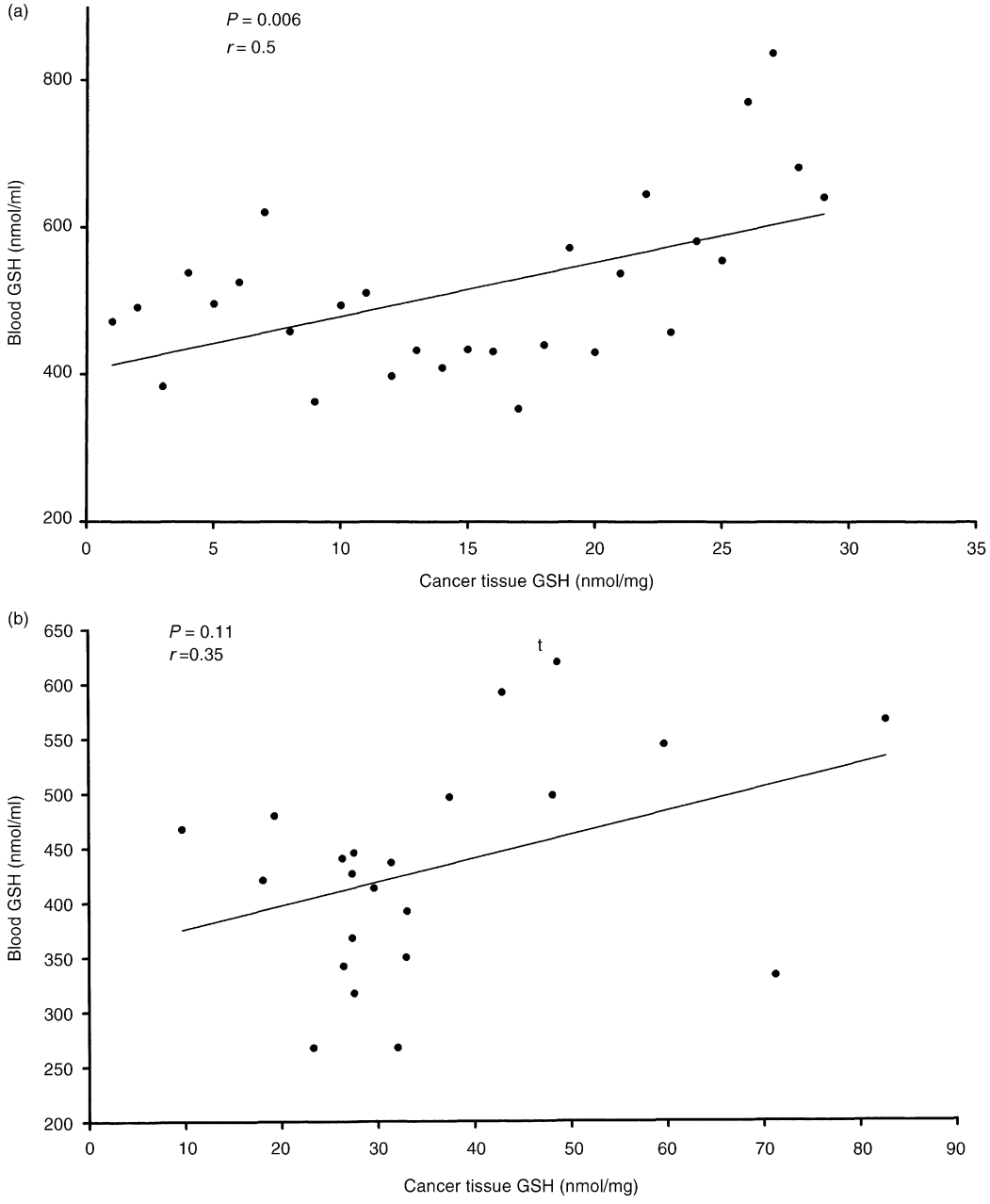

Fig. 2. Glutathione (GSH) level: correlation between tissue and blood levels: (a) non-small cell lung cancer (NSCLC) samples; (b) head and neck squamous cell carcinoma (SCCHN) samples.

method to be undetectable in 11/21 NSCLC and 3/15 SCCHN patients.

As far as tissue GST activity is concerned, this was measured in 29/29 cytosol samples of NSCLC and in 22/23 SCCHN patients, in both the cancer and matched normal tissue samples. As reported in Table 3, in NSCLC patients, the mean GST activity was 1.72 U/mg (S.D. = 0.89 U/mg) and 1.12 U/mg (S.D. = 0.43 U/mg) in cancer and normal tissues, respectively. In SCCHN, the mean GST activity was 2.61 U/mg (S.D. = 1.74 U/mg) and 2.07 U/mg (S.D. = 1.07 U/mg) in the cancer and normal tissues, respectively.

In NSCLC, the GST activity was significantly higher in the tumour samples than in the corresponding normal tissues ($P = 0.0002$). A weak difference ($P = 0.02$) between the tumour and normal tissue samples was also found in the SCCHN (Fig. 3). GST activity in the tumour was significantly correlated to GST activity in the normal tissue samples in both malignancies ($P = 0.01$, $r = 0.46$ in NSCLC, $P = 0.02$, $r = 0.48$ in SCCHN, Fig. 4a and b, respectively).

3.4. Correlation between GST activity and GSH level

The levels of GSH and GST activity in the cancer tissue were strongly correlated in NSCLC ($P < 0.0001$, $r = 0.7$) (Fig. 5) but not in SCCHN ($P = 0.07$, $r = 0.39$) (data not shown). In addition, a significant correlation

Table 3
Summary of the GST activity results

Variable	Cancer tissue (U/mg prot)	Normal tissue (U/mg prot)
Overall		
Mean	2.10	1.52
S.D.	1.38	0.90
Median	1.72	1.28
10–90th percentile	1.01–3.31	0.63–2.57
n	51	51
NSCLC		
Mean	1.72	1.12
S.D.	0.89	0.43
Median	1.37	1.10
10–90th percentile	0.87–2.97	0.62–1.76
n	29	29
SCCHN		
Mean	2.61	2.07
S.D.	1.74	1.07
Median	2.49	2.02
10–90th percentile	1.08–4.08	0.74–3.98
n	22	22

prot, protein.

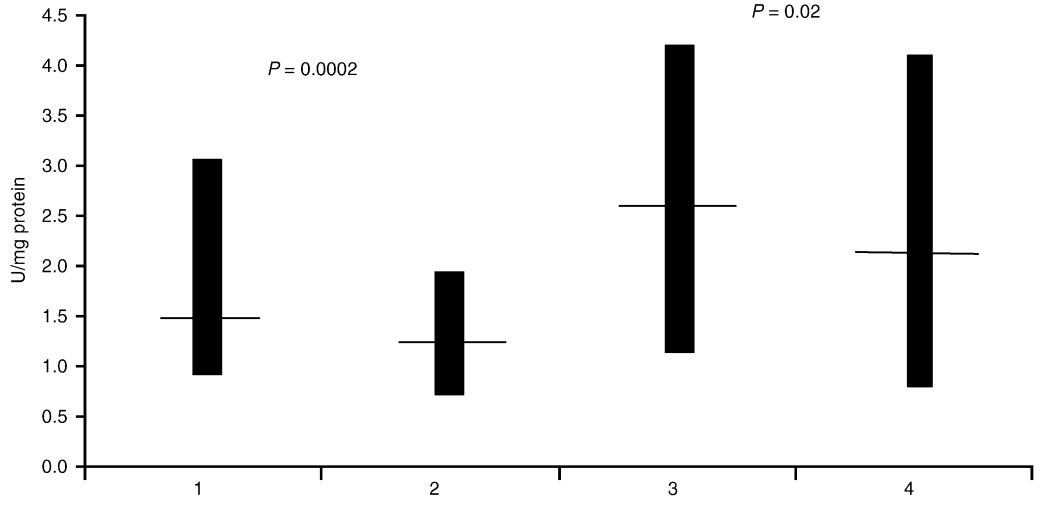

Fig. 3. Comparison of glutathione S-transferase (GST) activity level between tumour and normal tissues in non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (SCCHN) cancers. 1 = lung tumour tissue; 2 = lung normal tissue; 3 = head and neck tumour tissue; 4 = head and neck normal tissue.

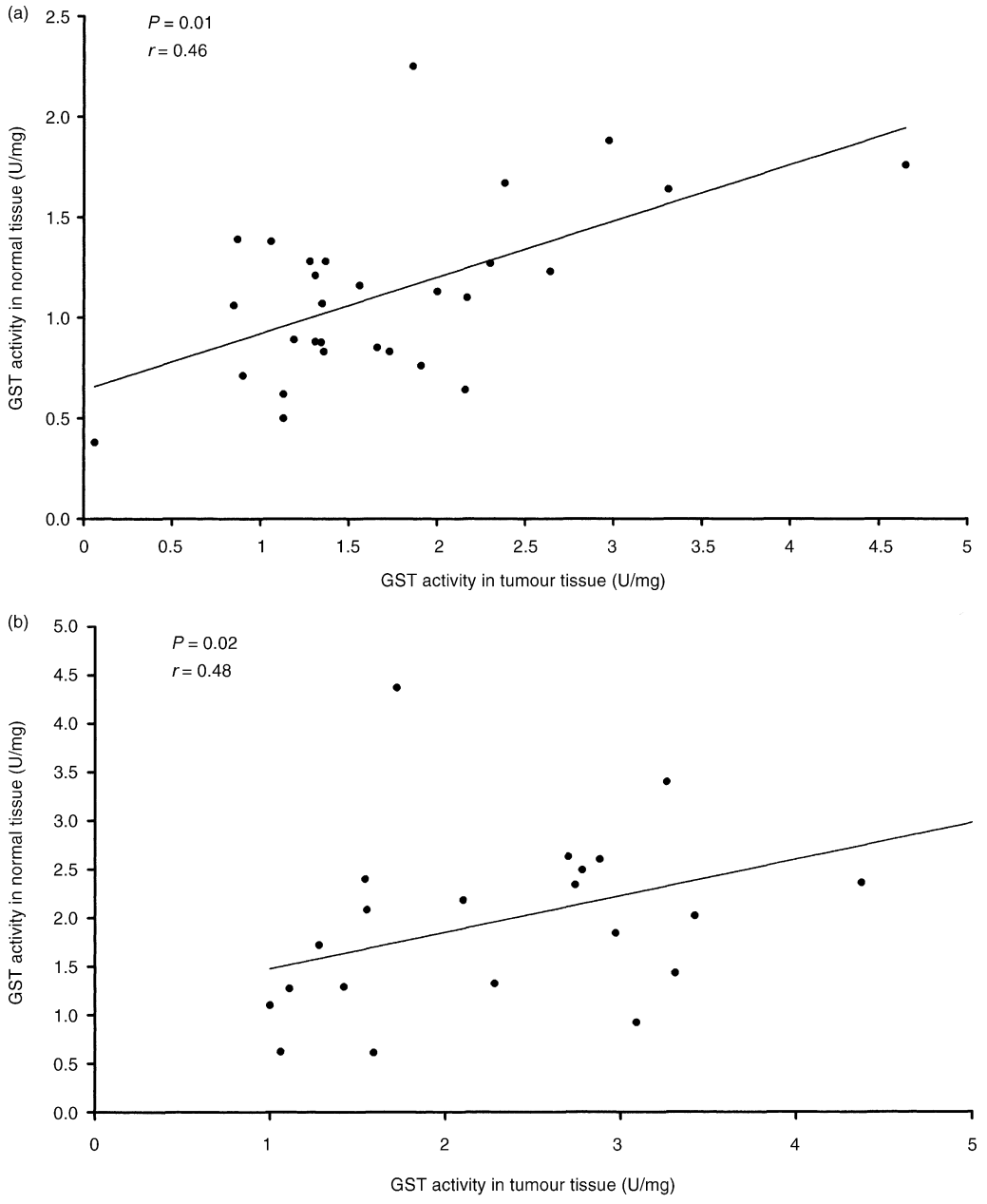

Fig. 4. Glutathione S-transferase (GST) activity level: correlation between tumour and normal tissues: (a) non-small cell lung cancer (NSCLC) samples; (b) head and neck squamous cell carcinoma (SCCHN) samples.

between GST activity in the cancer tissue and GSH level in whole blood was observed for both malignancies ($P=0.003$, $r=0.53$ in NSCLC, $P<0.0001$, $r=0.89$ in SCCHN, Fig. 6a and b, respectively). All the correlations are summarised in Table 4.

4. Discussion

Despite the fact that GST and GSH are known to modify both *in vitro* and *in vivo* sensitivity to many antitumour agents, there are several conflicting studies regarding the relationship between tissue and blood GSH and/or GST and the patient's response to therapy. This might reflect the difficulty in evaluating GST/GSH in biological tissues. Some of the difficulties may be ascribed to the existence of several analytes, i.e. GSH, GST activity and GST quantity. In addition, different GST isoenzymes may be investigated. Moreover, quite a few methods of GST detection have been described, including high performance liquid chromatography (HPLC), Western blot, immunochemistry, ELISA and GST activity assay [26]. Most of these methods are not standardised or fully validated and are often 'in-house'

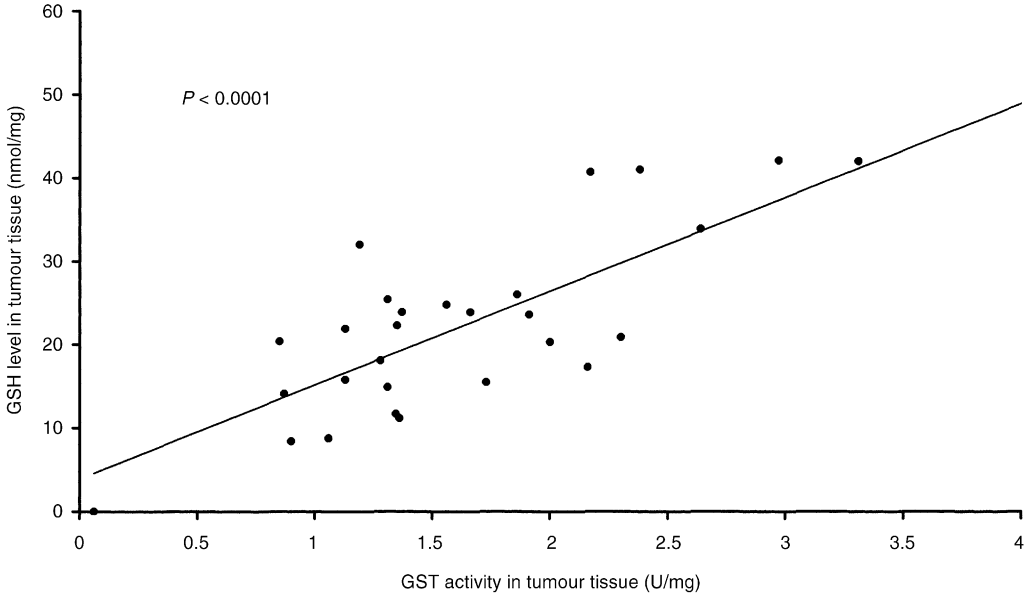

Fig. 5. Correlation between glutathione S-transferase (GST) activity and glutathione (GSH) level in non-small cell lung cancer (NSCLC) tissue.

Table 4
Summary of all correlations

	SCCHN				NSCLC			
	GST cancer tissue	GST normal tissue	GSH cancer tissue	GSH normal tissue	GST cancer tissue	GST normal tissue	GSH cancer tissue	GSH normal tissue
GST normal tissue	$P=0.02$ $r=0.48$	–			$P=0.01$ $r=0.46$	–		
GST cancer tissue	$P=0.07$ $r=0.39$	$P=0.80$ $r=0.06$	–		$P<0.0001$ $r=0.70$	$P=0.003$ $r=0.50$	–	
GSH normal tissue	$P=0.29$ $r=0.24$	$P=0.01$ $r=0.90$	$P=0.58$ $r=0.13$	–	$P=0.4$ $r=0.16$	$P=0.000$ $r=0.60$	$P=0.14$ $r=0.28$	–
GSH blood	$P<0.0001$ $r=0.89$	$P=0.01$ $r=0.50$	$P=0.11$ $r=0.35$	$P=0.39$ $r=0.40$	$P=0.003$ $r=0.53$	$P=0.02$ $r=0.40$	$P=0.006$ $r=0.50$	$P=0.55$ $r=0.11$

methods. However, methods for GSH detection are more limited as they include only HPLC or enzymatic assay. These methods are largely used in literature.

Finally, the studies addressing the clinical relevance of GSH/GST have frequently been performed on small series of patients with mixed malignancies at different stages, thus increasing the variability of results (i.e. primary tumours, untreated advanced disease, advanced disease under therapy).

No standardised method is currently available for GSH/GST measurement in humans. The aim of the present study was to validate some routinely feasible assays for GSH and GST measurement in biological tissues, as well as to explore the existence of any correlation between these two parameters. In particular the identification of a blood surrogate marker of cancer tissue GST/GSH expression could guide the selection of treatment for individual patients when already available DNA alkylating agents or novel compounds such as brostallicin, a DNA minor groove binder whose activity is enhanced by high levels of GSH and GST [27–29] are considered.

GSH was detected in all tumour and normal tissue samples from both NSCLC and SCCHN patients. GSH concentration was higher in tumour than in normal tissues, and the differences were more evident in NSCLC than in SCCHN. This observation is in agreement with some previous studies on patients with different tumours [30–32]. The bigger differences between normal and cancer tissues observed in NSCLC in comparison to SCCHN may reflect diverse cancer characteristics and/or different surgical techniques. Although these findings may be related to a divergent marker expression in the two types of malignancies, it is also possible that squamous histology may account for these differences. However, this point could not be investigated due to the

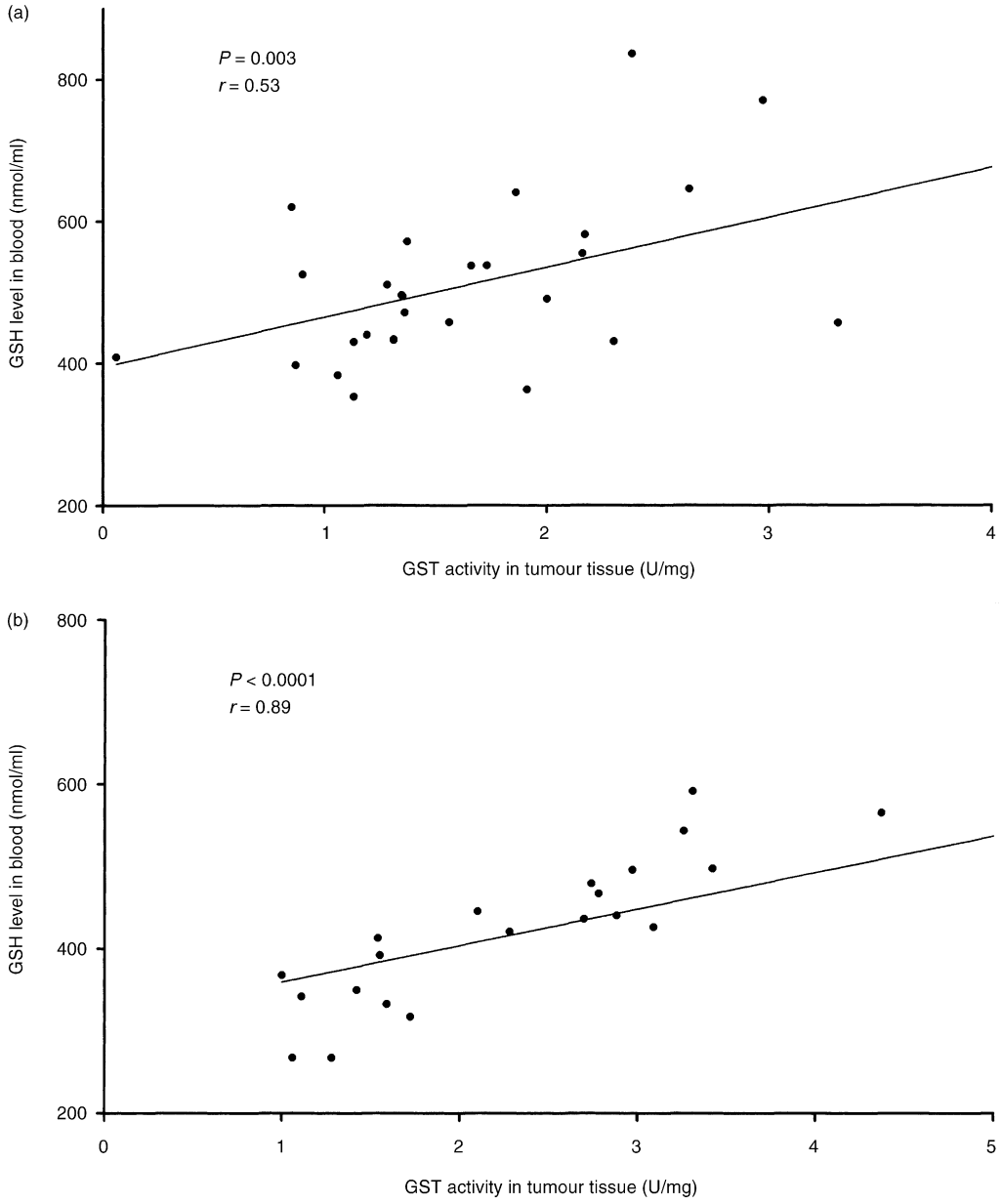

Fig. 6. Correlation between glutathione S-transferase (GST) activity in cancer tissue and glutathione (GSH) level in blood: (a) non-small cell lung cancer (NSCLC) samples; (b) head and neck squamous cell carcinoma (SCCHN) samples.

small number of squamous cell cases in the lung cancer group. Indeed, in NSCLC the normal tissue was obtained at a distance from the tumour, while in SCCHN the normal tissue was close to the cancer lesion. Moreover, SCCHN involves a multistep tumorigenesis process where premalignant alterations are frequently observed in areas adjacent to neoplastic regions [33]. This possible biological pattern which was not evaluated in the present study could bias the biological meaning of the markers value in the normal tissue samples of SCCHN.

GSH levels were not assayed in plasma or serum samples because of the low levels of GSH in these matrices. The assay protocol of the kit included a preliminary step of lyophilisation of plasma sample. Unfortunately, this procedure requires a larger amount of sample and lyophilisation is time-consuming and cumbersome, and therefore not suitable for routine application. However, GSH was detectable in all of the whole blood samples. GSH levels in the cancer tissues were significantly correlated with whole blood GSH levels in NSCLC, but not in SCCHN. To the best of our knowledge, this is the first report that examines the correlation between the GSH level in blood and tissue from the same patient.

We also measured GST activity in 22/23 of the tissue samples examined, and it was higher in the cancer than in normal tissues for both malignancies. As in the GSH

analysis, differences between cancer and normal tissues were larger in NSCLC than in SCCHN patients.

The observation of higher GST levels in cancer than in normal tissues was in agreement with the results of other studies in various malignancies such as stomach, colon, breast and ovarian cancers [16,34,35].

In plasma, GST activity was very low and close to the sensitivity threshold of the method. The data presented in literature are almost exclusively on plasma GST-Pi concentration and they are rather conflicting [36,37], probably because of the lack of strict sampling protocols. In fact, the delay in separation of plasma or the use of inadequate centrifugation (i.e. low speed centrifugation, room temperature centrifugation), are associated with marked increases in the plasma concentration of GST, probably as a result of the release of platelet GST into plasma [38].

However, Kura and colleagues [39] showed that GST-Pi is released in extracellular fluids and in plasma from platelets and from cancer cells in a monomeric form instead of the typical dimeric intracellular form. They also find that the monomeric form did not show enzymatic activity and these data could explain the low plasma GST activity we measured in our study.

In NSCLC tumour tissue samples, GST activity and GSH level seem to be higher in stage 1 than in the advanced stages. As mentioned above, given the limited number of available cases in each stage, any hypothesis to explain these results would be speculative. In tissue samples, GST activity and GSH level were significantly correlated in NSCLC, but not in SCCHN. In contrast, blood GSH levels were highly correlated with GST activity in tissues of both malignancies.

These findings suggest that the circulating levels of GSH may reflect GST status in NSCLC and SCCHN tumour tissues. In NSCLC, this observation is also supported by a highly significant correlation between both the GSH level and GST activity in tumour tissue on the one hand and GSH levels in cancer tissue and blood. However, in SCCHN no correlation was observed either between GSH levels in cancer tissue and blood, or GST activity and GSH level in cancer tissue. The reason for this difference remains to be investigated.

We can therefore conclude that the enzymatic method for the determination of GSH concentration in tissue extracts and blood is reliable, robust and suitable for routine use. GST activity can also be measured by enzymatic assay in tissues and the method is reliable, robust and suitable for routine use. In contrast, the sensitivity of the GST activity assay is not adequate for plasma samples.

In agreement with other authors, we have observed that both GST activity and GSH levels are higher in cancer than in normal tissues; however, this is the first time that GSH levels in whole blood show a highly significant correlation with GST activity in matched cancer samples. The use of GSH blood levels as a circulating surrogate marker of the tissue activity of the GST/GSH system should be further investigated. Although the number of cases in this study is limited, the preliminary results are encouraging. A study with a larger series of patients with different tumours is necessary to confirm these results.

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