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Pattern of S100-release in benign and malignant diseases beside malignant melanoma

Freisetzung von S100 bei benignen und malignen Erkrankungen jenseits des malignen Melanoms

Abstract

Background: The usefulness of S100 as a prognostic marker and aid in follow-up care in patients with malignant melanoma as well as in individuals with various neurological pathologies is well known. The aim of this study was to investigate its release and clinical relevance in benign and malignant disorders beyond these indications to elucidate tumor and organ specificity of S100.

Methods: S100 levels were studied in serum samples of 1856 untreated patients, among them 59 healthy individuals, 358 patients with benign disorders, and 1439 patients with malignant tumors.

Results: Healthy individuals had low S100 levels reaching a median of 0.041 ng/mL and 95th and 100th percentiles of 0.096 ng/mL and 0.144 ng/mL, respectively. The medians of patient groups with benign diseases ranged from 0.030 to 0.057 ng/mL, patients with malignant diseases from 0.020 to 0.059 ng/mL, and thus were comparable to healthy individuals. Only 2% of patients with benign diseases, mainly suffering from infectious, autoimmune, or benign gastrointestinal diseases, and 1% of patients with malignant diseases showed slightly higher values than healthy individuals, in most cases up to 0.5 ng/mL.

Conclusions: In contrast to many other oncological biomarkers, S100 is only rarely released in elevated levels from most benign and malignant diseases apart from malignant melanoma and neurological diseases, resulting in superior organ and tumor specificity. As potentially influencing factors, severe infectious diseases have to be considered.

Keywords: cancer; diagnosis; serum; S100.

Zusammenfassung

Hintergrund: S100 ist als nützlicher Biomarker für die Prognoseabschätzung und die Verlaufsbeobachtung bei Patienten mit malignem Melanom und in Patienten mit verschiedenen neurologischen Erkrankungen anerkannt. Das Ziel dieser Studie war, die Freisetzung und klinische Relevanz von S100 bei anderen benignen und malignen Erkrankungen jenseits dieser Indikationen zu untersuchen, um die Tumor- und Organspezifität von S100 zu bewerten.

Methoden: S100-Konzentrationen wurden in Serumproben von 1856 unbehandelten Patienten ermittelt, darunter 59 gesunde Personen, 358 Patienten mit benignen Erkrankungen und 1439 Patienten mit malignen Tumoren.

Ergebnisse: Gesunde Personen hatten niedrige S100 Serumwerte, die einen Median von 0.041 ng/mL, eine 95. Perzentile von 0.096 ng/mL und ein Maximum von 0.144 ng/mL erreichten. Die Mediane der Patientengruppen lagen bei benignen Erkrankungen zwischen 0.030 und 0.057 ng/mL, bei Patienten mit malignen Tumoren zwischen 0.020 und 0.059 ng/mL – und waren somit vergleichbar zu gesunden Kontrollpersonen. Lediglich 2% der Patienten mit benignen Erkrankungen, v.a. infektiösen, autoimmun oder benignen gastrointestinalen Erkrankungen, sowie 1% der Patienten mit malignen Tumoren wiesen im Vergleich zu gesunden Personen leicht erhöhte Werte auf – in den meisten Fällen bis 0.5 ng/mL.

Schlussfolgerung: Im Gegensatz zu vielen anderen onkologischen Biomarkern wird S100 nur selten in höheren Konzentrationen von den meisten benignen und malignen Erkrankungen – mit Ausnahme des malignen Melanoms und neurologischer Erkrankungen – freigesetzt, was sich in einer sehr hohen Organ- und Tumorspezifität widerspiegelt. Als möglicher Einflussfaktor sind schwere infektiöse Erkrankungen zu berücksichtigen.

Schlüsselwörter: Diagnose; Krebs; Serum; S100.

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Introduction

S100 constitutes a thermolabile, acidic protein of 21 kDa molecular weight which was originally detected in the central nervous system of vertebrates [1–3]. Its name derives from its biochemical properties to remain soluble even in 100% ammonium sulfate at neutral pH. S100 is part of a multigenic family of calcium-binding proteins and consists of a dimer of two isomeric subunits (α , 10.4 kDa and β , 10.5 kDa). It exists in the isoforms S100B ($\beta\beta$), S100A ($\alpha\beta$), and S100A1 ($\alpha\alpha$) [1–3].

S100B is localized in high concentrations in astroglial cells of the central nervous system; in addition, it is produced in lower amounts by Schwann cells of the peripheral nervous system, by chondrocytes, adipocytes, and Langerhans cells. S100A is expressed particularly by malignant melanoma cells and shows a correlation with invasivity and tumor extent. In the central nervous system, S100A is present in low amounts contributing only 5% to total cerebral S100 concentration. S100A1 is found in keratinocytes, melanocytes, in smooth muscle cells, cardiomyocytes, and in the kidney [1–3].

Currently, 21 isoforms of the S100 family are known. Owing to their function as intracellular calcium receptor molecules, they are involved in the regulation of the cell cycle at various levels such as differentiation and proliferation. Additionally, S100 interacts with tumor suppressor protein p53 and blocks its phosphorylation by protein kinase C. As a result, p53 is not able to maintain its function in regulating the cell cycle, DNA repair, and induction of apoptosis [1, 2]. Furthermore, S100B is known to bind to the receptor for advanced glycation end products (RAGE) and leads among others to activation of astrocytes, which are involved in various neurological

disorders [4]. Stimulation of RAGE is further reported as a central mechanism of proinflammatory proteins S100A8/A9 [5] and S100A6 [6]. Interestingly, S100B and S100A6 subtypes bind to different extracellular RAGE domains and modulate cell survival and proliferation by different RAGE-dependent pathways [6].

In malignant melanoma, S100 is widely appreciated for immunohistochemical diagnosis and is used in serum for differential diagnostic purposes of lymph node positivity or metastatic disease [1, 2, 7–9]. In addition, it has been proposed for detection of metastatic uveal melanoma [10, 11]. Furthermore, it is an independent predictive and prognostic marker and is recommended for progression detection in advanced stages and for the monitoring of systemic therapies in patients with and without distant metastases [1, 2, 12–21]. Thereby, S100B has been shown to have superior prognostic information than lactate dehydrogenase (LDH) [7, 16, 22, 23] and to be additive to melanoma inhibitory antigen (MIA) [7, 24]. In addition, S100 in serum and cerebrospinal fluid is valuable for the diagnosis and follow-up of neurodegenerative and neurodegenerative diseases, such as cerebral trauma, ischemia and infection, in cases of hypoxic lesions after cardiac arrest or interventions with cardiopulmonary bypass. Particularly after acute cerebral events, the determination of S100 in serum is appropriate for the estimation of the disintegration of the blood-brain barrier [3, 25, 26].

Most concurrent commercially available S100 assays use mono- or polyclonal antibodies directed against the β -subunit of S100 and detect the isoforms S100A and S100B [27–29]. However, some assays also enable more detailed characterization of S100 subtypes [17]. Comparisons between different methods for the quantification of S100 revealed good assay characteristics for automatized assays, however, often only moderate to poor correlations with considerable slopes, particularly to manual assays [27–29]. In the present study, S100 values were assessed by the automatized Elecsys® S100 assay (Roche Diagnostics, Mannheim, Germany), which has shown excellent methodical performance in earlier studies [28, 29].

In the past tumor markers were often only validated for diagnosis, prognosis, and/or therapy monitoring in those tumor entities for which the parameter revealed a particular sensitivity, and the influence of other benign and malignant diseases on the release of this tumor marker was not considered. However, because these interfering factors would be of high relevance, particularly for the interpretation of kinetic observations during or after therapy, we evaluated this S100 assay according to the recommendations of the European Group on Tumor Markers

(EGTM) [30] beyond the classical applications to elucidate its tumor and organ specificity.

Patients and methods

In total, we investigated serum samples of 1856 individuals including 59 healthy persons, 1439 patients with various cancer diseases (lung, colorectal, breast, ovarian, cervix-uterus, stomach, hepatocellular, prostate, pancreatic, bladder, and others), and 358 with various benign diseases relevant for differential diagnosis (gastrointestinal, gynecological, lung, breast, urological, prostate, autoimmune, infections, and others) (Table 1). All samples were obtained at the time of acute disease and before the start of the recommended therapy – in cancer patients mostly before surgery.

For determination of S100, we used the Elecsys® S100 assay working with two monoclonal mouse antibodies directed against the S100 β -subunit. In this system, 20 μ L of the patient sample is incubated with a biotinylated S100 antibody and a ruthenium-labeled S100 antibody forming a sandwich complex. After adding streptavidin-coated microparticles, this complex binds to the solid phase, which is subsequently transferred to the detection cell. By magnetic effects, the microparticles are fixed on the surface of an electrode, and after a

Table 2 Sensitivities of detecting cancer disease at a 95% specificity to patients with the relevant benign comparison group.

Group	Sensitivity, %	Cut-off value, ng/mL
Lung cancer	2	0.096
Colorectal cancer	1	0.121
Breast cancer	24	0.068
Ovarian cancer	2	0.092
Cervical cancer	7	0.094
Gastric cancer	10	0.121
Hepatocellular cancer	10	0.121
Prostate cancer	6	0.095
Pancreatic cancer	3	0.121
Bladder cancer	10	0.072

washing step they can be quantified by determination of chemiluminescence emission by a photomultiplier. By use of a two-point calibration and a master curve, the concentration of S100 in the sample is calculated. The lower detection limit is indicated at an analytical sensitivity level of <0.005 ng/mL, the functional sensitivity is <0.02 ng/mL.

Distribution of the values is shown by dot plots and statistical tables. Sensitivities for the various malignant diseases were calculated using the 95% percentile of the relevant benign comparison group as a cut-off value (Table 2).

Table 1 Distribution of S100 values in healthy persons and patients with various benign and malignant diseases (number, median, range, 95% percentile).

Group	n	Median, ng/mL	Range, ng/mL	95% Percentile, ng/mL
Healthy individuals	59	0.041	0.001–0.144	0.096
Benign gastrointestinal disease	50	0.051	0.015–0.223	0.161
Benign gynecological disease	50	0.057	0.028–0.105	0.100
Benign lung disease	50	0.050	0.016–0.186	0.147
Benign breast disease	50	0.037	0.010–0.084	0.072
Benign urological disease	52	0.036	0.001–0.115	0.088
Benign prostate hyperplasia	29	0.045	0.011–0.110	0.102
Autoimmune disease	20	0.030	0.001–0.388	0.373
Infections	30	0.052	0.018–1.960	1.069
Lung cancer	472	0.020	0.001–0.301	0.062
Colorectal cancer	682	0.022	0.001–0.534	0.064
Breast cancer	50	0.049	0.003–0.193	0.163
Ovarian cancer	50	0.042	0.006–0.106	0.091
Cervical cancer	27	0.032	0.002–0.236	0.187
Gastric cancer	30	0.035	0.001–0.373	0.350
Hepatocellular cancer	30	0.059	0.022–0.462	0.320
Prostate cancer	29	0.044	0.013–0.256	0.184
Pancreatic cancer	30	0.046	0.006–0.171	0.129
Bladder cancer	30	0.036	0.001–0.156	0.156

Results

S100 values in serum of healthy persons were found to be very low with a median of 0.041 ng/mL, a 95% percentile of 0.096 ng/mL, and a range between <0.005 ng/mL and 0.144 ng/mL (Figure 1, Table 1). In patients with benign disorders, S100 values were comparable with medians between 0.030 ng/mL and 0.052 ng/mL (Figure 2, Table 1). The ranges of S100 values were in most

disorders between <0.005 ng/mL and 0.25 ng/mL. Only some patients with autoimmune diseases reached values up to 0.40 ng/mL; and most importantly, in single cases of patients with acute infections values were observed up to 1.96 ng/mL. Patients with various malignant diseases did not show markedly elevated levels of S100 (Figure 3, Table 1). The medians were for most cancers in the range of benign diseases (0.030–0.059 ng/mL). The highest S100 values were observed in colorectal cancer with 0.534 ng/mL,

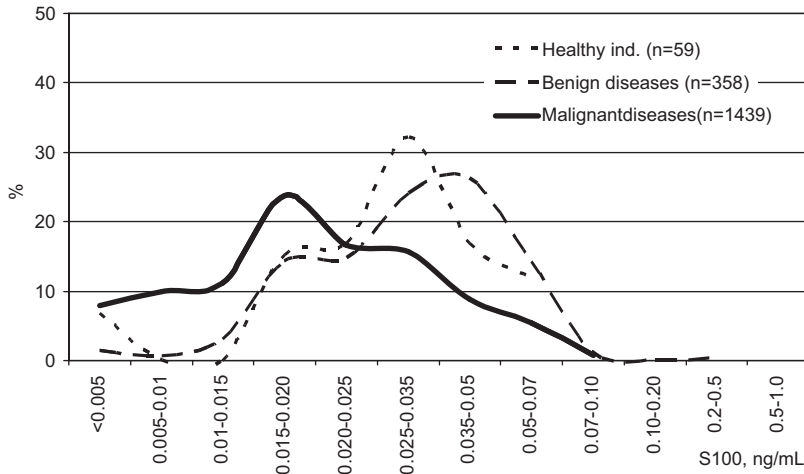


Figure 1 Frequency distribution of S100 values in healthy persons, in patients with benign diseases, and in those with malignant diseases.

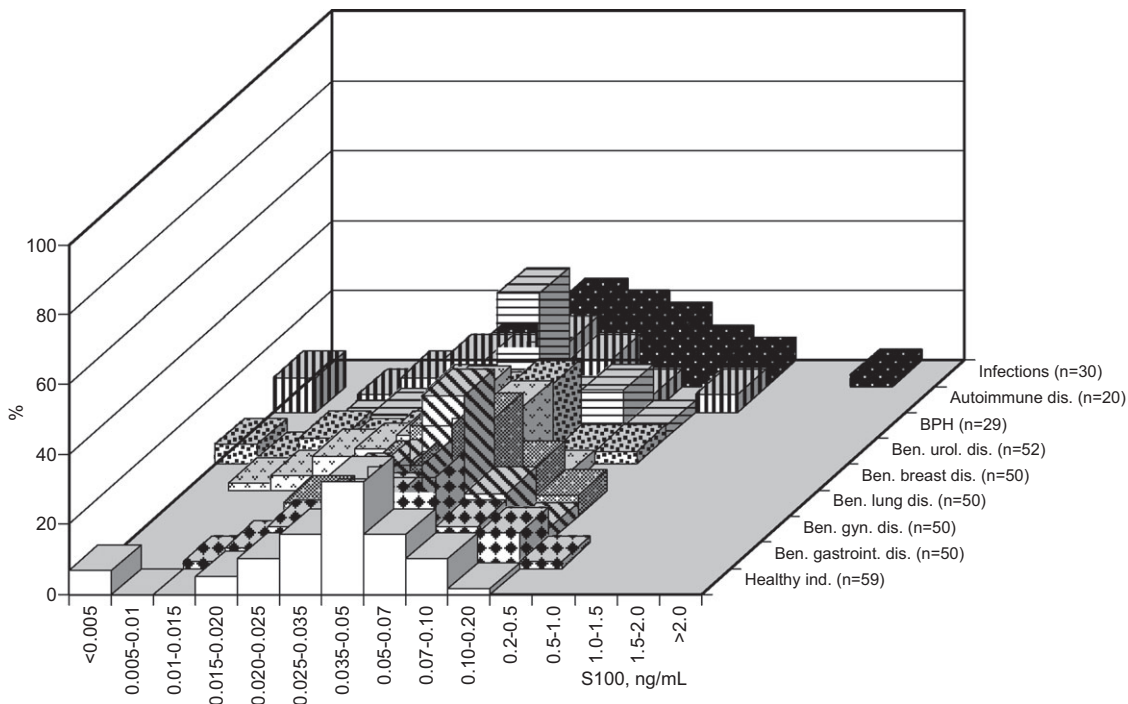


Figure 2 Distribution of S100 values in healthy persons and patients with various benign diseases.

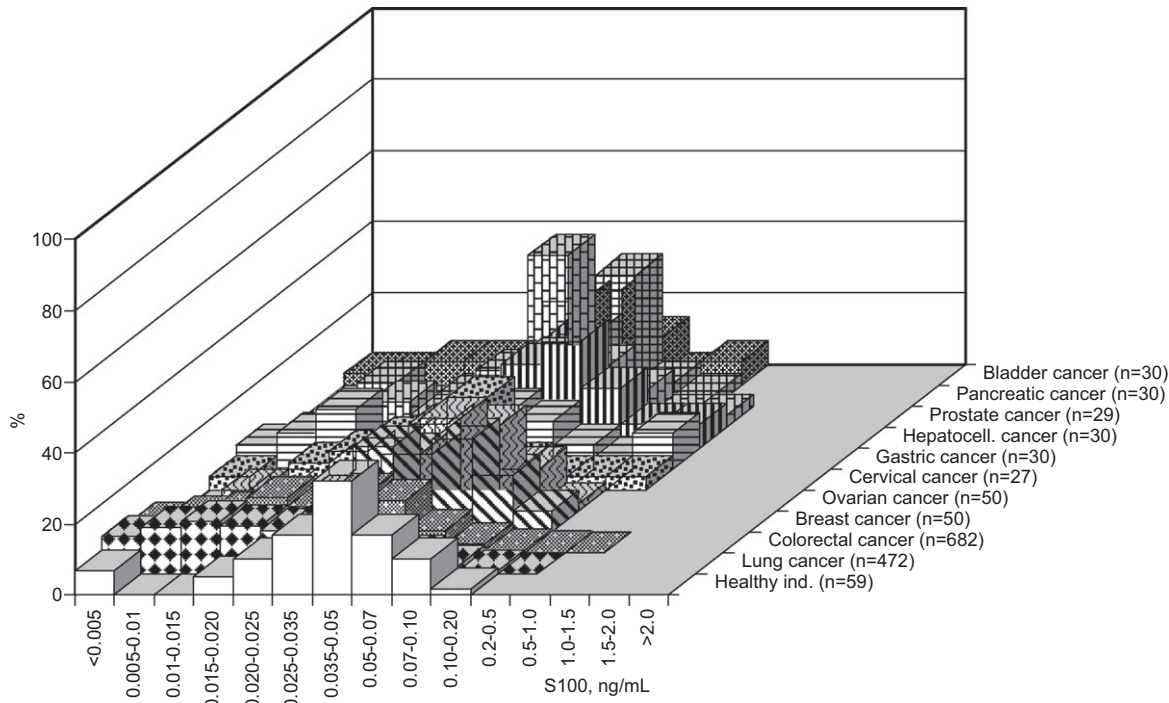


Figure 3 Distribution of S100 values in patients with various malignant tumors.

and in hepatocellular cancer with 0.462 ng/mL. Concerning the sensitivity for cancer detection, cut-off values were defined at the 95% percentile of the relevant benign comparison group. For most tumors investigated, the sensitivities ranged up to 10%. Only in breast cancer, a sensitivity of 24% was observed (Table 2).

Discussion

Several studies have shown the value of S100 in diagnosis, prognosis, and therapy monitoring of malignant melanoma. Expression and release of S100 were observed to be elevated in serum of patients with malignant melanoma with clear correlation to stage. Whereas during stages I and II, the median of serum S100 values was comparable with the median of healthy individuals, stage III and particularly stage IV disease were associated significantly with higher S100 levels [1, 31–36]. In one study including advanced stages of melanoma, a median of 6.25 ng/mL and maximum values >90 ng/mL were reported [31]. Concerning the site of distant metastases, liver and bone metastases were related to higher S100 values than cutaneous lesions [33, 36].

In addition to diagnostic aspects, S100 was found to be a valuable prognostic marker concerning progression-free interval and overall survival [18–23, 35–40]. In 670

high-risk surgically resected melanoma patients, high baseline and increasing S100 values were independent prognostic markers [20]. Also in stages IIb and III melanoma patients, S100 was of high prognostic value [19, 37]. In stage IV, S100 strongly correlated with LDH [36] and had superior prognostic value than LDH in multiple settings [7, 16, 22, 23]. The clinical relevance of S100 as a prognostic marker in melanoma patients independent from TNM stage was confirmed by a recent meta-analysis on more than 3000 patients [18], which emphasized its prognostic role particularly for stage I–III patients.

Serial determination of S100 proved to be useful for longitudinal observations during or after treatment. Particularly in stage III, progression was detected with a lead time of 5 to 23 weeks prior to the appearance of macroscopic metastases [41–43]. In stage IV, S100 increases were correlated with insufficient responses to systemic therapy. In some cases, a see-saw pattern was observed with strong decreases after every application followed by increases until the next treatment date [32]. By contrast, efficient therapy resulted in significant decreases of S100 to low levels at the detection limit [12, 42–44]. Recently, S100 was also found to be useful to assess response to bevacizumab induction treatment in stage III melanoma patients [13]. In a follow-up of melanoma patients, S100 showed to be valuable for early detection of progression as an adjunct to positron emission computed tomography, particularly in asymptomatic patients [14].

Similar to many other biochemical blood parameters, S100 concentrations in serum might be influenced by pathophysiological conditions that affect the expression, release, or metabolism of S100 in circulation. These disorders include various benign and malignant diseases that would be crucial to identify when S100 levels in serum have to be interpreted, particularly for therapy monitoring or detection of recurrent disease in melanoma patients. For example, earlier studies have shown that elevated serum concentrations of S100 are present in patients after trauma, cerebral ischemia and infections, hypoxic lesions after cardiac arrest, and after surgical heart interventions with cardiopulmonary bypasses being involved [3, 25, 45–48].

To elucidate the tumor and organ specificity of S100 for malignant melanoma, we investigated in detail various benign diseases that are known to potentially alter the concentrations of several tumor markers in serum such as benign gastrointestinal, gynecological, lung, breast, and urological diseases, benign prostate hyperplasia, as well as autoimmune and infectious diseases, including also those with renal and hepatic failure [49]. In addition, we addressed various malignant tumor diseases that might confound S100 levels in serum and be relevant for differential diagnosis of suspicious lesions.

In healthy persons, most studies have found only low serum S100 concentrations with a median of 0.04 ng/mL and a 95% percentile of approximately 0.1 ng/mL, which is in concordance with our results. Differences according to gender and age were not observed [50]. However, benign disorders have been investigated in detail only by one group using a luminimetric S100 assay. Corresponding with our findings, S100 levels in many benign pulmonary, gynecological, urological, gastrointestinal, and autoimmune diseases were reported to be in the range of healthy persons. Only liver cirrhosis and renal failure was related to slightly higher S100 values up to 0.7 ng/mL; in addition, one patient with renal failure exhibited strongly

elevated levels of 4.75 ng/mL [31]. In our evaluation, we could not confirm these findings. However, we found that in some cases of bacterial infections S100 values were up to 1.96 ng/mL. Particularly, in polytraumatic patients at intensive care units and those being at risk of septic complications, these results have to be considered for differential diagnosis.

As expected, most malignant disorders did not lead to substantial elevations of serum S100. No patients with breast, ovarian, cervix, gastric, liver, pancreatic, bladder, and prostate cancers showed higher S100 values. Only single cases were observed with concentrations up to 0.53 ng/mL. These observations confirm that, unlike many other oncological biomarkers, high serum concentrations of S100 (>0.5 ng/mL) have a high specificity for malignant melanoma, which is relevant for the differential diagnosis of a cancer of unknown primary (CUP), particularly if suspicious cerebral, hepatic, or pulmonary lesions are present. Owing to its high tumor and organ specificity, S100 is, further, a valuable marker for therapy monitoring and detection of recurrent disease in melanoma patients. However, potentially influencing factors have to be considered for interpretation of S100 kinetics.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Provision of the reagents played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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