# Serum Carbohydrate Sulfotransferase 7 in lung Cancer and Non-malignant Pulmonary Inflammations

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**Abstract**

**Background:** Carbohydrate sulfotransferases (CHST) were shown to be involved in carcinogenesis. The aim of the study was to assess the diagnostic value of serum CHST7 concentration in differentiation between lung cancer and non-malignant pulmonary inflammations.
**Methods:** Clinical case-control study involving 125 participants was conducted: the control group containing cases of pneumonia and chronic obstructive pulmonary disease was compared to the lung cancer group composed of primary and metastatic cancers. Serum concentrations of CHST7 and routinely used markers including carcinoembryonic antigen (CEA), cytokeratin fragment 21-1 (CYFRA 21-1) and neuron-specific enolase (NSE) were determined for each participant using immunochemical methods. Statistical association, receiver operating characteristic (ROC) analysis and cross-validation were used for the evaluation of CHST7 either as a standalone biomarker or as a part of a biomarker panel.
**Results:** In comparison to the control group, serum CHST7 was elevated in lung cancer ($p < 0.001$), but no differences between the overall stages of primary cancers were detected ($p = 0.828$). The differentiation performance in terms of ROC area under curve (AUC) was 0.848 making CHST7 superior biomarker to the NSE ($p = 0.031$). In comparison to CEA and CYFRA 21-1, the performance differences were not detected. CHST7 was not correlated to other biomarkers, and its addition to the routine biomarker panel significantly improved the cross-validated accuracy (85.6% vs. 75.2%) and ROC AUC ($p = 0.004$) of the differentiation using a machine learning approach.
**Conclusions:** Serum CHST7 is a promising biomarker for the differentiation between lung cancer and non-malignant pulmonary inflammations.

**Keywords:** biomarker; carbohydrate sulfotransferase; CHST7; lung cancer.

## Introduction

Hyaluronan-binding chondroitin sulfate proteoglycans (CSPG) like versican together with CD44 membrane receptor and the hyaluronan itself play an important role in different physiological processes like cell migration and recognition, extracellular matrix (ECM) deposition and morphogenesis [1–7], which are critically involved in the carcinogenesis [8–14]. Due to the well-described role in carcinogenesis and metastatic processes, hyaluronan, versican and CD44 are routinely used diagnostic and prognostic parameters of the lung cancer, among others. Biosynthesis of functional forms of versican and CD44 critically depends on the sulfation of chondroitin chains catalyzed by the carbohydrate (chondroitin) sulfotransferase (CHST) enzymes, CHST7 in particular. The fact that CHST7 controls biosynthesis of CSPG involved in the carcinogenesis and metastatic processes shifts the focus from the hyaluronan, versican and CD44 to the CHST7.

To the best of our knowledge, the diagnostic properties of CHST7 have not been evaluated yet in any clinical context. Before engaging in a more elaborate clinical study, we briefly evaluated the hypothesized association of CHST7 with the most common type of lung cancer, i.e. non-small cell lung carcinoma (NSCLC). Preliminary

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evaluation involved the univariate statistical analysis of publicly available microarray data sets containing CHST7 expressions determined in peripheral blood mononuclear cells (PBMC) and lung tissue samples collected in the clinical case-control studies [15–17]. This analysis revealed the significant differences in PBMC CHST7 expressions between NSCLC and non-malignant pulmonary diseases (results not shown). Moreover, the significant differences in CHST7 expressions were also detected when the malignant tissue samples were compared to the surrounding non-malignant lung tissue samples of NSCLC patients setting grounds for the clinical evaluation of CHST7.

The described properties and preliminary results classify CHST7 in the category of lung cancer biomarker candidates. Moreover, the intracellular localization of CHST7 implies that increased cellular destruction, which occurs in malignancies, may be reflected in the elevation of serum CHST7 concentration, which opens the possibility for development of non-invasive applications. Non-invasive and widely available yet reliable differentiation of the lung cancer from other pulmonary diseases presenting with pulmonary inflammation still represents an elusive goal [18–20]. This is especially important problem in the chronic obstructive pulmonary disease (COPD) case [21–25]: lung cancer and COPD are tightly associated and frequently coexist, but they require different therapeutic approaches. In an attempt to tackle this problem, the clinical evaluation of serum CHST7 enzyme-linked immunosorbent assay (ELISA) used for the differentiation between lung cancer and non-malignant pulmonary inflammations has been selected to be the object of this study.

# Materials and methods

## Clinical study design

Evaluation of serum CHST7, i.e. index test used for the differentiation of lung cancer from non-malignant pulmonary inflammations, has been designed as the clinical case-control study, which was conducted in Osijek University Hospital, Croatia, and University Hospital Center Zagreb, Department for Lung Diseases Jordanovac, Croatia, in the period from 2012 to 2015. Where applicable, REMARK and STARD guidelines [26, 27] were followed. Patients of both gender, older than 18 years, admitted to a hospital due to the suspected malignant pulmonary disease accompanied with clinical and laboratory signs of inflammation, who gave the consent to participate, were eligible for the study. We chose the systematic, consecutive sampling of patients according to the order of their admission to a hospital. Subjects suffering from the non-pulmonary malignancies like lymphomas and pleural mesotheliomas were excluded from the study same as subjects for which the diagnostic workup was incomplete. All participants enrolled in the study were chemotherapy naïve. Of 125 participants, 71 were primary or metastatic (secondary) lung cancer cases and 54 were cases of non-malignant pulmonary inflammations. A detailed description of the studied population is given in the Table 1 and Figure 1. This study has been conducted in accordance with the World Medical Association Declaration of Helsinki 2013 [28]. All enrolled subjects have signed informed consent, and the study has been granted approval by the Ethical Committees of both hospitals.

**Table 1:** Clinical characteristics of pulmonary patients enrolled in the study.

| Properties/cases | Lung cancer (no. of cases) | Non-malingnant inflammation (no. of cases) |
|---|---|---|
| Habits | | |
| Smoker | 37 | 11 |
| Ex smoker | 29 | 12 |
| Non-smoker | 5 | 31 |
| Inflammatory conditions | | |
| COPD | 23 | 28 |
| Pneumonia | – | 23 |
| Others | – | 3 |
| Cancer histology | | |
| Adenocarcinoma | 31 | – |
| Squamous cell carcinoma | 20 | – |
| Large cell carcinoma | 1 | – |
| NSCLC (not otherwise specified) | 2 | – |
| Small cell lung carcinoma (SCLC) | 7 | – |
| Carcinoid | 2 | – |
| Metastatic (secondary) cancer | 8 | – |
| Gender | | |
| Male | 55 | 33 |
| Female | 16 | 21 |
| Age, years | | |
| Median (range) | 64 (19–90) | 70 (35–89) |

Blood samples taken from all enrolled subjects were used for the determination of serum CHST7 and routinely used diagnostic panel of lung cancer biomarkers including serum carcinoembryonic antigen (CEA), cytokeratin fragment 21-1 (CYFRA 21-1) and neuron-specific enolase (NSE). The routinely used serum biomarkers served as a benchmark for comparisons of diagnostic performance and statistical association studies. In addition to the comparison between cases and controls, CHST7 concentrations were compared across different cancer stages and different lung tumors. The heterogeneity of molecular signatures of pulmonary malignancies and underlying etiologies, which are frequently overlapping with other lung diseases, urges the use of cumulative diagnostic approach that assumes simultaneous application of different tools, including different biomarkers. The influence of the serum CHST7 on the cumulative diagnostic performance of the lung cancer biomarker panel was evaluated by the multivariate differentiation using machine learning. For that purpose, the routine biomarker panel performance was compared to the performance of the panel extended by the CHST7.

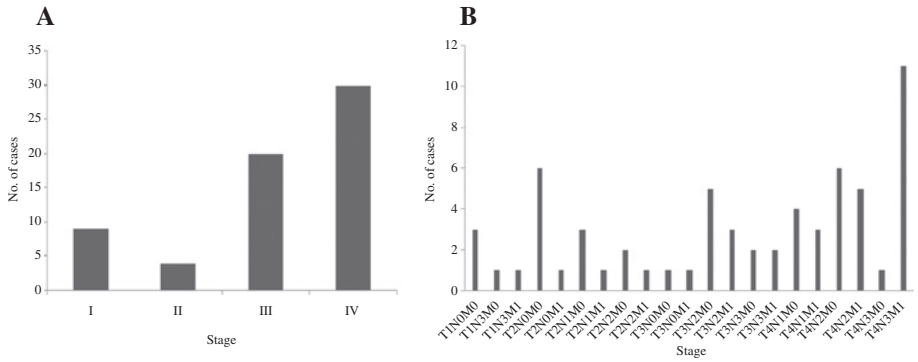


**Figure 1:** Distribution of the primary lung tumor cases according to stages.
(A) Overall (prognostic) staging. (B) TNM staging (according to the TNM classification of malignant tumors, 7th edition).

### Imaging diagnostics

The diagnostic workup of each participant included spirometry, radiographic imaging techniques, bronchoscopy with samples collection accompanied by cytological and/or histological analysis. Radiographic imaging used in this study composed of chest radiography in the anterior-posterior and lateral view and the multislice computer tomography of thorax and upper abdomen. Based on the listed procedures, lung cancer diagnosis was confirmed or excluded.

### Laboratory evaluation

Cancer types were established using cytological and histological samples according to the World Health Organization classification of lung tumors [29]. The tumor-node-metastasis (TNM) staging system was used for the clinical staging of malignant cases [30].

Blood samples were collected in the sample tubes containing no anticoagulant (Becton Dickinson, Plymouth, UK). Within 1 h after collection, blood samples were centrifuged at 1300*g* for 10 min. Obtained serum samples were aliquoted, frozen and kept at –70 °C until the analysis. Serum CHST7 concentrations (μg/L) of the undiluted samples were determined using ELISA (USCN Life Science Inc., Wuhan, China) implemented on ETI-Max 3000 (DiaSorin, Saluggia, Italy). Concentrations of CEA, CYFRA21-1 and NSE were measured using the electrochemiluminescence immunoassay implemented on COBAS E 601 analyzer (Roche Diagnostics, IN, USA). Electrochemiluminescence immunoassays were validated by the manufacturer, and their performances have been regularly evaluated by the internal and external quality assessment schemes. CHST7 ELISA performances were evaluated using the internal quality assessment materials provided by the kit manufacturer.

### Statistical analysis

Mann-Whitney U (MWU) test was applied for the unmatched pairwise comparisons. This test was conducted using the statistical package Statistica 7.0 (Stat Soft, Inc., Tulsa, OK, USA). Together with well-known diagnostic parameter performance metrics, the difference in receiver operating characteristic (ROC) area-under-curve (AUC), i.e. Δ ROC AUC, has been used in this study. This metric is defined as a simple difference between the ROC AUC values, which correspond to two competing diagnostic parameters. As the ROC AUC itself, Δ ROC AUC is also a test statistic, which means that statistical significance p could be assigned to it. In this case, p represents the statistical significance of difference between the competing diagnostic parameter performances. ROC AUC and differences thereof were calculated by the DeLong's method [31–33], whereas confidence intervals (CI) were calculated by the bootstrap method involving 10,000 replicates. The minimum distance between ROC curve and upper left corner of the ROC graph was used for the serum CHST7 threshold establishment required for sensitivity and specificity analyses. The multivariate differentiation of malignant cases from non-malignant controls has been conducted using the random forest (RF) machine/statistical learning approach (default settings were used) [34, 35]. Leave-one-out (LOO) cross-validation ROC of the multivariate differentiation was used as the estimator of cumulative diagnostic performance. Pearson's correlation, ROC and RF computations were conducted using the statistical package R version 3.1.2. (R Foundation for Statistical Computing, Vienna, Austria) [36]. Results were considered significant if the corresponding p-value was <0.050.

# Results

## Association studies

Box-and-whisker plots showing serum CHST7 distributions in lung cancer and non-malignant pulmonary inflammations are given in Figures 2 and 3. Differences in the serum CHST7 concentrations between the overall lung cancer group and non-malignant inflammation group were statistically significant ($n = 125$, $p < 0.001$). All pairwise comparisons of non-malignant inflammations with each of the histological types of lung tumors (Figure 2) and with each of the overall (prognostic) stages of primary lung tumors have shown statistically significant differences ($p < 0.050$) in the serum CHST7 concentrations.

Serum CHST7 concentrations of the cancer group or group of coexisting cancer and COPD were significantly increased in pairwise comparison with the COPD

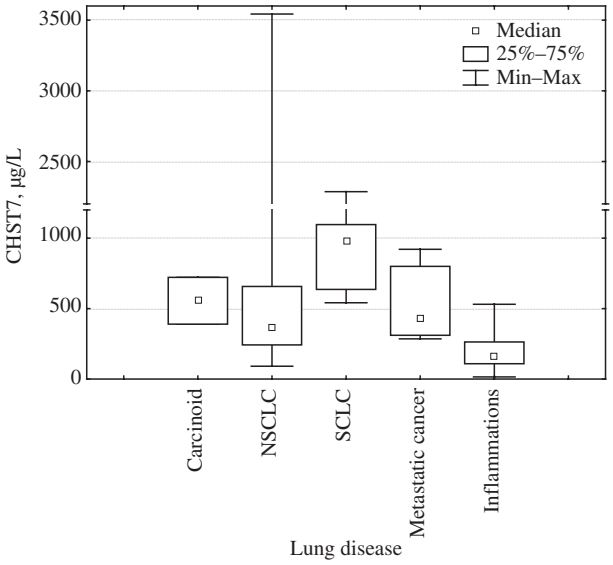


**Figure 2:** Serum CHST7 in lung cancer subtypes and non-malignant inflammations.

group and to the group involving other inflammations (Figure 3): $p < 0.001$ was calculated in all mentioned cases. No differences in the serum CHST7 concentration have been detected between the genders ($n = 125$, $p = 0.212$) or between the localized (T1-4N0M0) and the metastatic (T1-4N1-3M0-1) primary lung tumors ($n = 63$, $p = 0.846$). In case of TNM staging, the only significant difference in serum CHST7 was detected between the T classes of primary tumors (Figure 4): in comparison to T2 and T3 classes, the T4 class was associated with

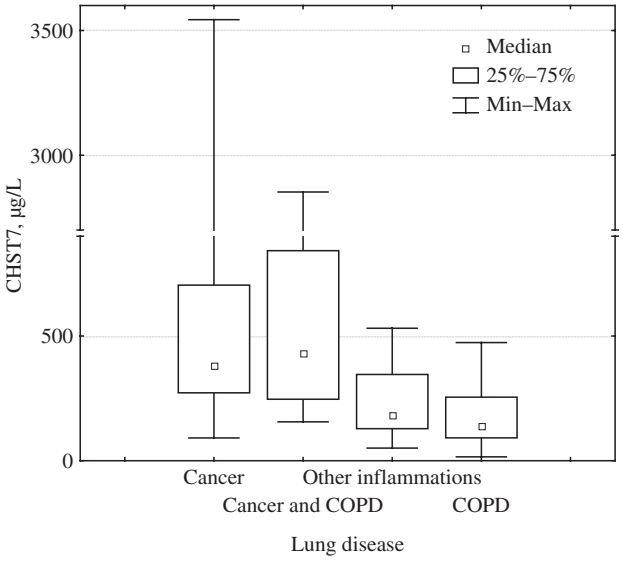


**Figure 3:** Serum CHST7 in patients suffering from lung cancer, coexisting lung cancer and COPD, COPD alone and other pulmonary inflammations.

significantly higher CHST7 concentration ($p = 0.007$, $n = 34$ and $p = 0.004$, $n = 34$, respectively).

## Differentiation between lung cancer and non-malignant pulmonary inflammations by serum CHST7

### Univariate diagnostic performance of serum CHST7 and routine biomarkers

CHST7 performance and its comparison to the univariate performances of routine lung cancer markers are given in Tables 2 and 3 and Figure 5. Actual CHST7 ROC AUC and Δ ROC AUC between CHST7 and routine markers were used as the performance estimators.

### Effect of CHST7 on the cumulative diagnostic performance of lung cancer biomarker panels

Multivariate differentiation of lung cancer from the non-malignant pulmonary inflammations conducted by the

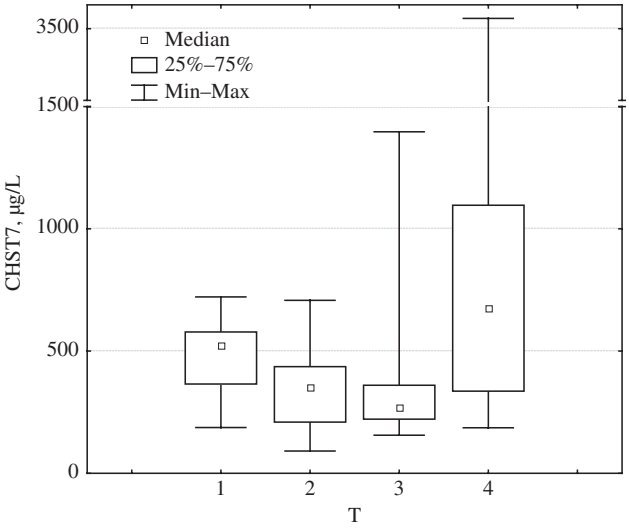


**Figure 4:** Serum CHST7 in different T classes of primary lung tumors.

**Table 2:** ROC of serum CHST7 used for the differentiation between lung cancer and non-malignant pulmonary inflammations ($n = 125$).

| Diagnostic property | |
|---|---|
| AUC (95% CI) | 0.848 (0.775–0.909) |
| Sensitivity (95% CI) | 0.778 (0.667–0.889) |
| Specificity (95% CI) | 0.747 (0.648–0.845) |

Optimal threshold for the differentiation was 268 µg/L.

**Table 3:** Differentiation between lung cancer and non-malignant pulmonary inflammations (n = 125): routine biomarkers' ROC AUC, univariate ROC AUC differences (Δ ROC AUC) and correlations between the serum CHST7 and routine biomarkers.

| Routine biomarker | ROC AUC | Δ ROC AUC (p) | Pearson's correlation (p) |
|---|---|---|---|
| CEA | 0.749 | 0.099 (0.077) | 0.020 (0.824) |
| CYFRA 21-1 | 0.824 | 0.024 (0.657) | 0.055 (0.546) |
| NSE | 0.727 | 0.121 (*0.031*) | 0.138 (0.124) |

Value in italics indicates significant results.

RF has been evaluated using the LOO cross-validation (n = 125): cross-validated Δ ROC AUC (Figure 5B) was 0.088 (p = 0.004), and it favors extended (CHST7, CEA, CYFRA21-1, NSE) over the routine lung cancer biomarker panel (CEA, CYFRA21-1, NSE). The actual LOO classification accuracies were 85.6% and 75.2%, respectively.

## Discussion

In comparison to non-malignant pulmonary inflammations, lung cancer is characterized by the substantially elevated serum CHST7 concentration (Figure 2). Figure 2 reveals that this is a property shared by all pulmonary malignancies where the small cell lung carcinoma (SCLC) shows the highest elevation of serum CHST7. Elevated CHST7 expression and/or decay of the malignant tissue, including stromal cells like macrophages, may at least partially explain the described elevation of serum CHST7 in all types of lung cancer: CHST7 expression in macrophages rises in response to the interleukin 4 (IL-4) stimulation, which is a general characteristic of lung malignancies [10, 37]. In contrast to the IL-4, interferon γ (IFNγ) stimulation that is a characteristic of the non-malignant inflammations reduces CHST7 expression in the tissue macrophages, maintaining low serum CHST7 concentration. However, CHST7 may be expressed in other cells like peripheral immune cells or cancer cells, and the serum CHST7 elevation in lung malignancies requires further investigation. CHST7 that is elevated in both primary and metastatic (secondary) pulmonary malignancies (Figure 2) reinforces the hypothesis that ties the serum CHST7 elevation to immune cells, stromal and/or peripheral remains to be determined.

All overall (prognostic) stages of primary lung tumors were associated with the significantly increased serum CHST7, and this elevation was uniform i.e. no significant differences among the stages were detected. TNM stages of primary lung tumors were also associated with the roughly uniform serum CHST7 elevation: even when localized (T1-4N0M0) and metastatic (T1-4N1-3M0-1) primary lung tumors were compared, no differences were found. The only significant difference among the tumor stages in terms of serum CHST7 was detected when the T classes were compared (Figure 4). Serum CHST7 exceptionally elevated in the T4 stage suggests that the invasion of large vessels or surrounding organs was the main cause of exceptional CHST7 elevation as opposed to the lymphatic nodes involvement and the existence of distant metastases: serum CHST7 elevation in N stages and M stages was uniform. Invasion of surrounding organs and large vessels implies the extensive basement membrane remodeling

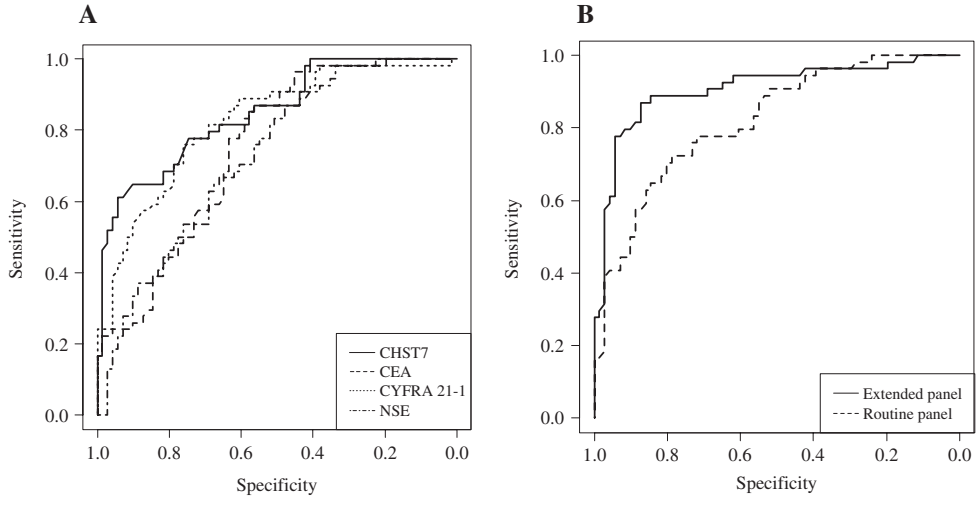


**Figure 5:** ROC analyses: differentiation between lung cancer and non-malignant pulmonary inflammations (n = 125).
(A) Univariate performance comparisons of CHST7 and routine lung cancer markers. (B) Cross-validated performance of routine panel (CEA, CYFRA 21-1, NSE) and the routine panel extended by CHST7 using RF multivariate classification (p = 0.004).

either as a part of the tumor dissemination or as a part of the angiogenesis. Immune cells and fibroblasts localized in the basement membrane meshwork composed of CSPG may be the source of CHST7 required for the extensive basement membrane remodeling distinctive to the T4 cases of primary lung cancer.

Highly significant results coming from association studies (Figure 2) paved the way for evaluation of serum CHST7 diagnostic performances. Serum CHST7 concentration used for the differentiation between lung cancer and non-malignant pulmonary inflammations performed equally or better than the routinely used lung cancer biomarkers (Tables 2 and 3 and Figure 5A): CHST7 is characterized by the highest ROC AUC of 0.848, but the difference in ROC AUC is statistically significant only in the NSE case. Lung cancer and pulmonary inflammations are the tightly associated diseases where inflammations either precede or coexist with the lung cancer. Table 2 and Figure 3 suggest that the serum CHST7 may be used in such situations making serum CHST7 a candidate biomarker for the COPD patients follow-up: in comparison to the COPD alone, the CHST7 tends to be elevated even when lung cancer and COPD coexist (Figure 3). The fact that there is no difference in CHST7 serum concentration between the overall (prognostic) stage I and later stages of primary lung cancers, all characterized by the increased serum CHST7, reinforces the proposed use of this enzyme: a marked elevation of CHST7 in COPD patients may be an early sign of the malignant transformation. Low to moderate elevations, however, should be evaluated carefully due to the limited specificity of this biomarker candidate, which is a common property shared by all serum biomarkers analyzed in this study (Table 2 and Figure 5A).

Differences in the underlying molecular signatures between lung cancer cases may be reflected in the different behaviors of studied biomarkers. Lack of correlation between the CHST7 and other lung cancer biomarkers (Table 3) is in line with this hypothesis, and it led to evaluation of CHST7 influence on the cumulative diagnostic performance of the biomarker panels. Expected improvement of the cumulative diagnostic performance achieved by the introduction of an independent parameter, namely serum CHST7, to the routine lung cancer biomarker panel has been confirmed, and it is highly significant (Figure 5B). Cross-validated accuracy of 85.6% corresponds to the extended panel containing only four lung cancer biomarkers of which three markers are routinely used. In order to achieve similar diagnostic accuracies for the differentiation between NSCLC and non-malignant lung diseases using microarrays, Showe et al. [17] devised a panel of 29 gene expression probes.

This study represents the first attempt to evaluate the diagnostic performance of serum CHST7 in any clinical context. It suffers from some disadvantages mainly associated with the enrollment of subjects based on order of admission. The distribution of cancer stages and the age of onset of pulmonary diseases reflect the cancer stage and age of cancer patients recruited from the Croatian population. In this population, the later stages of cancer predominate and cancer patients are slightly younger than the patients suffering from the non-malignant pulmonary inflammations. These properties of enrolled population may compromise some of the study outcomes. Future studies should address the question of serum CHST7 performance in settings involving early stages of lung cancer and case and control groups more equilibrated in terms of age, gender, smoking status, etc. Also, large cohorts are needed to comprehensively evaluate the relations between many TNM stages and histological types of lung cancer in terms of the serum CHST7.

## Conclusions

To the best of our knowledge, this is the first report on application of serum CHST7 in cancer diagnostics. In comparison to non-malignant pulmonary inflammations, the CHST7 concentration was elevated not only in all clinical stages and evaluated types of primary lung cancer but also in metastatic (secondary) lung cancer. ROC analyses conducted under the univariate settings showed that the differentiation performance of serum CHST7 is equally good or better than the performance of routine biomarkers. CHST7 values in COPD and lung cancer patients suggest that CHST7 is a candidate marker for COPD patients' diagnostic follow-up. Introduction of CHST7 to the panel containing only three routinely used biomarkers improved the differentiation of lung cancer from non-malignant pulmonary inflammations using machine learning: the cross-validated diagnostic accuracy was comparable to the accuracies achieved in similar settings by the gene expression microarrays. Investigation of the serum CHST7 determined by different analytical methods applied on samples taken from the larger and stratified populations involving participants with early stages of lung cancer is underway.

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**Author contributions:** ŽD, SD, BD and SB conceived and designed the experiments. SD, ŽD, SM, SB and MM performed the experiments. ŽD, SD, SM, SB, LM and MS analyzed the data. ŽD, VŠ, VB and MS coordinated the research. VŠ and VB contributed reagents, materials and analysis tools. ŽD, SM, SB and SD wrote the manuscript. MM, MS, BD and LM revised the manuscript. ŽD supervised the research. All authors read and approved the final manuscript.

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## References

1. Kitagawa H, Fujita M, Ito N, Sugahara K. Molecular cloning and expression of a novel chondroitin 6-O-sulfotransferase. J Biol Chem 2000;28:21075–80.
2. Cooney CA, Jousheghany F, Yao-Borengasser A, Phanavanh B, Gomes T, Kieber-Emmons AM, et al. Chondroitin sulfates play a major role in breast cancer metastasis: a role for CSPG4 and CHST11 gene expression in forming surface P-selectin ligands in aggressive breast cancer cells. Breast Cancer Res 2011;13:R58.
3. Cattaruzza S, Schiappacassi M, Kimata K, Colombatti A, Perris R. The globular domains of PG-M/versican modulate the proliferation-apoptosis equilibrium and invasive capabilities of tumor cells. FASEB J 2004;18:779–81.
4. Pirinen R, Leinonen T, Böhm J, Johansson R, Ropponen K, Kumpulainen E, et al. Versican in non-small cell lung cancer: relation to hyaluronan, clinicopathologic factors, and prognosis. Hum Pathol 2005;36:44–50.
5. Ly M, Laremore TN, Linhardt RJ. Proteoglycomics: recent progress and future challenges. OMICS 2010;14:389–99.
6. Stylianou M, Skandalis SS, Papadas TA, Mastronikolis NS, Theocharis DA, Papageorgakopoulou N, et al. Stage-related decorin and versican expression in human laryngeal cancer. Anticancer Res 2008;28:245–51.
7. Suwiwat S, Ricciardelli C, Tammi R, Tammi M, Auvinen P, Kosma VM, et al. Expression of extracellular matrix components versican, chondroitin sulfate, tenascin, and hyaluronan, and their association with disease outcome in node-negative breast cancer. Clin Cancer Res 2004;10:2491–8.
8. Tammi RH, Kultti A, Kosma VM, Pirinen R, Auvinen P, Tammi MI. Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan. Semin Cancer Biol 2008;18:288–95.
9. Wu YJ, La Pierre DP, Wu J, Yee AJ, Yang BB. The interaction of versican with its binding partners. Cell Res 2005;15:483–94.
10. Ruffell B, Poon GF, Lee SS, Brown KL, Tjew SL, Cooper J, et al. Differential use of chondroitin sulfate to regulate hyaluronan binding by receptor CD44 in inflammatory and interleukin 4-activated macrophages. J Biol Chem 2011;286:19179–90.
11. Pirinen R, Tammi R, Tammi M, Hirvikoski P, Parkkinen JJ, Johansson R, et al. Prognostic value of hyaluronan expression in non-small-cell lung cancer: increased stromal expression indicates unfavorable outcome in patients with adenocarcinoma. Int J Cancer 2001;95:12–7.
12. Vachon E, Martin R, Plumb J, Kwok V, Vandivier RW, Glogauer M, et al. CD44 is a phagocytic receptor. Blood 2006;107:4149–58.
13. Luo Z, Wu RR, Lv L, Li P, Zhang LY, Hao OL, et al. Prognostic value of CD44 expression in non-small cell lung cancer: a systematic review. Int J Clin Exp Pathol 2014;7:3632–46.
14. Ween PM, Oehler KM, Ricciardelli C. Role of versican, hyaluronan and CD44 in ovarian cancer metastasis. Int J Mol Sci 2011;12:1009–29.
15. Sanchez-Palencia A, Gomez-Morales M, Gomez-Capilla JA, Pedraza V, Boyero L, Rosell R, et al. Gene expression profiling reveals novel biomarkers in non-small cell lung cancer. Int J Cancer 2011;129:355–64.
16. Hou J, Aerts J, den Hamer B, van Ijcken W, den Bakker M, Riegman P, et al. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. PLoS One 2010;5:e10312.
17. Showe MK, Vachani A, Kossenkov AV, Yousef M, Nichols C, Nikonova EV, et al. Gene expression profiles in peripheral blood mononuclear cells can distinguish patients with non-small cell lung cancer from patients with nonmalignant lung disease. Cancer Res 2009;69:9202–10.
18. Scott A, Salgia R. Biomarkers in lung cancer: from early detection to novel therapeutics and decision making. Biomark Med 2008;2:577–86.
19. Qi W, Li X, Kang J. Advances in the study of serum tumor markers of lung cancer. J Canc Res Ther 2014;10:95–101.
20. Doseeva V, Colpitts T, Gao G, Woodcock J, Knezevic V. Performance of a multiplexed dual analyte immunoassay for the early detection of non-small cell lung cancer. J Transl Med 2015;13:55.
21. Durham AL, Adcock IM. The relationship between COPD and lung cancer. Lung Cancer 2015;90:121–7.
22. Takiguchi Y, Sekine I, Iwasawa S, Kurimoto R, Tatsumi K. Chronic obstructive pulmonary disease as a risk factor for lung cancer. World J Clin Oncol 2014;5:660–6.
23. Gonzalez J, Marin M, Sanchez-Salcedo P, Zulueta JJ. Lung cancer screening in patients with chronic obstructive pulmonary disease. Ann Transl Med 2016;4:160.
24. Punturieri A, Szabo E, Croxton TL, Shapiro SD, Dubientt SM. Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. J Natl Cancer Inst 2009;101:554–9.
25. Sekine Y, Katsura H, Koh E, Hiroshima K, Fujisawa T. Early detection of COPD is important for lung cancer surveillance. Eur Respir J 2012;39:1230–40.

26. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. Reporting recommendations for tumour MARKer prognostic studies (REMARK). Brit J Cancer 2005;9:387–91.
27. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Clin Chem 2015;61:1446–52.
28. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013;310:2194.
29. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JH, Beasley MB, et al. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 2015;10:1243–60.
30. Chheang S, Brown K. Lung cancer staging: clinical and radiologic perspectives. Semin Intervent Radiol 2013;30:99–113.
31. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
32. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77.
33. Sing T, Sander O, Beerenwinkel N, Lengauer T. ROCR: visualizing classifier performance in R. Bioinformatics 2011;21:3940–1.
34. Breiman L. Random forests. Mach Learn 2001;45:5–32.
35. Liaw A, Wiener M. Classification and regression by random forest. R News 2002;2:18–22.
36. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2014.
37. Quatromoni JG, Eruslanov E. Tumor-associated macrophages: function, phenotype and link to prognosis in human lung cancer. Am J Transl Res 2012;4:376–89.