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Inflammatory biomarkers and cancer: CRP and suPAR as markers of incident cancer in patients with serious nonspecific symptoms and signs of cancer

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In Denmark, patients with serious nonspecific symptoms and signs of cancer (NSSC) are referred to the diagnostic outpatient clinics (DOCs) where an accelerated cancer diagnostic program is initiated. Various immunological and inflammatory biomarkers have been associated with cancer, including soluble urokinase plasminogen activator receptor (suPAR) and the pattern recognition receptors (PRRs) pentraxin-3, mannose-binding lectin, ficolin-1, ficolin-2 and ficolin-3. We aimed to evaluate these biomarkers and compare their diagnostic ability to classical biomarkers for diagnosing cancer in patients with NSSC. Patients were included from the DOC, Department of Infectious Diseases, Copenhagen University Hospital Hvidovre. Patients were given a final diagnosis based on the combined results from scans, blood work and physical examination. Weight loss, Charlson score and previous cancer were registered on admission, and plasma concentrations of biomarkers were measured. The primary outcome was incident cancer within 1 year. Out of 197 patients included, 39 patients (19.8%) were diagnosed with cancer. Patients with cancer were significantly older and had a higher burden of comorbidities and previous cancer diagnoses compared to patients who were not diagnosed with cancer. Previous cancer, C-reactive protein (CRP) and suPAR were significantly associated with newly diagnosed cancer during follow-up in multiple logistic regression analyses adjusted for age, sex and CRP. Neither any of the PRRs investigated nor self-reported weight loss was associated with cancer. In this study, previous cancer, CRP and suPAR were significantly associated with cancer diagnosis in patients with NSSC. Ficolin-1-3, MBL and pentraxin-3 were not associated with cancer.

Cancer is the leading cause of death in Denmark. To reduce diagnostic delay and improve survival, between 2007 and 2012, the Danish Health Authority introduced standardized fast-track outpatient cancer pathways. A pathway was designed for patients with serious nonspecific symptoms and signs of disease that could be cancer (NSSC) and patients with metastasis without a known primary tumor or organ-specific manifestation. These patients undergo an accelerated pathway of cancer diagnostics at

Key words: diagnosis, neoplasms, ficolin, PTX3 protein, mannose-binding lectin

Abbreviations: AUC: area under the curve; CI: confidence interval; CRP: C-reactive protein; DOC: diagnostic outpatient clinic; ESR: erythrocyte sedimentation rate; IQR: interquartile range; MBL: mannose-binding lectin; NAD: no abnormality detected; NMD: nonmalignant disease; NPV: negative predictive value; NSSC: nonspecific symptoms and signs of cancer; PPV: positive predictive value; PRR: pattern recognition receptor; ROC: receiver operating characteristics; SD: standard deviation; suPAR: soluble urokinase plasminogen activator receptor; WBC: white blood cell.

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What's new?

In Denmark, patients with serious non-specific symptoms and signs of cancer are referred to an accelerated cancer diagnostic program. But screening and diagnosis is challenging as patients form a heterogeneous group. Possible advances in the diagnostic strategy must be studied to improve accuracy and safety. This study found that the novel inflammatory biomarker suPAR and the routinely evaluated inflammatory biomarker CRP were independently associated with incident cancer diagnoses, while the innate immune markers, pentraxin-3, mannose-binding lectin and ficolin-1-3, were not. Addition of suPAR to the existing blood samples may improve diagnosis and prognostication of cancer in this heterogeneous patient group.

the diagnostic outpatient clinics (DOCs). The prevalence of cancer diagnosed at Danish DOCs is 16–20%.[1–3] The remaining patients are found to have a wide range of nonmalignant diseases, but 25–50% of the patients leave the DOCs without a specific diagnosis.[1,2]

Screening and diagnosis is challenging as patients referred to the DOCs form a heterogeneous group. Possible advances in the diagnostic strategy must be studied to improve accuracy and safety when examining patients suspected of having serious disease.

Patients are characterized by different nonspecific complaints, such as weight loss, fatigue, diffuse abdominal or bone pain, anemia or an increase in drug usage or more frequent contacts with the health care system.[1] The initial diagnostic process at the DOC includes a focused medical history, a physical examination, application of an expanded panel of biochemical analyses and, if malignancy remains suspected, imaging is performed.[3]

Research has recently reported various immunological and inflammatory serum biomarkers associated with different types of cancer that could constitute new diagnostic or prognostic markers in the development of cancer and prognosis.[4–6]

Fluid-phase pattern recognition receptors (PRRs) of the innate immune system, including pentraxin-3, mannose-binding lectin (MBL), ficolin-1, ficolin-2 and ficolin-3, recognize pathogens and modified self-molecules and have been found to be associated with cancer.[7–17] Another novel biomarker in this setting is the soluble urokinase plasminogen activator receptor (suPAR) which reflects inflammatory activity. suPAR is the soluble form of uPAR which, in cancer, is expressed on cancer cells and stromal cells.[18–20] Several studies show an association between suPAR and the presence and prognosis of different types of cancer[21–27] as well as an increased risk of developing lung cancer in the general population.[28]

Little is known as to how these biomarkers perform in a heterogeneous group of patients with diffuse and nonspecific symptoms. Therefore, we evaluated these novel biomarkers and compared their diagnostic ability to that of classical biomarkers for diagnosing cancer in patients with NSSC.

Materials and Methods

Study design and patient inclusion

The current study of biomarkers in the DOC was conducted as part of a study evaluating two different diagnostic imaging modalities, ^{18}F-FDG-PET/CT *vs*. conventional CT of the thorax and abdomen.[3]

Patients were prospectively included from the DOC, Department of Infectious Diseases, Copenhagen University Hospital Hvidovre between August 14, 2013, and April 30, 2014. Patients were referred from their general practitioner, a medical specialist or other hospital departments. The criteria for referral to the DOC were suspicion of serious illness or suspicion of metastasis without a known primary tumor, based on one or more of the following symptoms present that do not fit into any of the organ-specific cancer diagnostic programs: general malaise, severe tiredness, unintentional weight loss, fever of unknown cause, uncharacteristic abdominal pain for >4 weeks, anemia, abnormal laboratory tests (*e.g.*, elevated alkaline phosphatase levels, erythrocyte sedimentation rate (ESR), calcium etc.), diffuse bone pain, pathologically enlarged lymph nodes, marked increase in drug usage or increasing health service seeking behavior.

Inclusion criteria for the study were *i*) age ≥18 years, *ii*) referred to the DOC due to nonspecific symptoms or signs of cancer and *iii*) signed informed consent. Exclusion criteria were *i*) pregnancy, including risk of pregnancy and lactation, *ii*) alcohol or drug abuse hampering the ability to adhere to the protocol, *iii*) claustrophobia, *iv*) bodyweight above 150 kg, *v*) contraindications to CT due to allergy to contrast or impaired renal function defined as P-creatinine level >0.120 mmol/L or *vi*) deemed unfit due to performance status.

Randomization and clinical evaluation was performed as previously described.[3] Briefly, at the first visit to the DOC, patients were randomized 1:1 to be scanned with either conventional CT or ^{18}F-FDG-PET/CT. The randomization was based on a computer-generated list (GraphPad Software, Inc., La Jolla, CA) and was performed by a study nurse blinded to the patient's medical history, prior to any laboratory testing. Experienced certified radiologists and nuclear medicine physicians analyzed the CT or ^{18}F-FDG-PET/CT data and discussed the results with the clinicians. Furthermore, patients went through a physical examination including evaluation of biochemistry. The combined results from scans, blood work and physical examination provided the basis for the diagnostic decisions made by the DOC physicians at a multidisciplinary conference with the participation of specialist physicians from the following medical specialties: endocrinology, gastroenterology, nuclear medicine, radiology and infectious diseases. A preliminary tentative

diagnosis was given here, and a final referral diagnosis was given when the full investigational program for disease at the DOC was complete. If a malignant diagnosis was established, the patients were referred to further treatment.

Patients were followed for 12 months after finishing their investigations in the DOC by reviewing the electronic medical records, for confirmation of malignant diagnoses based on pathology results from biopsies etc.

Measurements

Weight loss and previous cancer diagnoses were self-reported measures, registered in the electronic medical records at the first visit to the DOC.

Albumin, CRP, hemoglobin, lactate dehydrogenase, ESR and white blood cell count were analyzed as part of the routine evaluation at the Department of Clinical Biochemistry, Copenhagen University Hospital Hvidovre.

Plasma suPAR levels were analyzed with the suPARnostic AUTO Flex ELISA (ViroGates A/S, Birkerød, Denmark) on an automated Siemens BEP2000 platform at the Department of Clinical Biochemistry according to the manufacturer's instructions.

Plasma concentrations of ficolin-1, ficolin-2, ficolin-3, pentraxin-3 and MBL were determined by sandwich ELISAs using specific in house-produced monoclonal antibodies as previously described.[29–33] All assays were optimized for automated analysis in 384-well format on Biomek FX (Beckman Coulter, Fullerton, CA).

The Charlson comorbidity index (Charlson score) was assessed at the first consultation at the DOC by reviewing the patient's medical record.

Outcomes and covariates

The primary outcome was any incident cancer diagnoses within 1 year.

Multivariate analyses were adjusted for age and sex, and furthermore, as CRP is a widely used marker of inflammation in the clinic, we included CRP in the adjusted analyses.

Statistical analysis

Continuous variables are presented as mean and standard deviation (SD) or median and interquartile range (IQR). Categorical variables are presented as number and percentage.

Differences between groups were tested with *t*-test, Fisher's exact test or the Wilcoxon two-sample test where appropriate.

Associations were analyzed by multiple logistic regression. For these analyses, weight loss was categorized in groups per 5 kg weight loss, hemoglobin was log(1.1)-transformed and albumin, lactate dehydrogenase, CRP, ESR, suPAR, ficolin-1, ficolin-2, ficolin-3, MBL and pentraxin-3 were log(2)-transformed.

Kendall's Tau-b correlation coefficient was reported for pairwise correlation analyses for CRP, ESR and suPAR.

Biomarker discriminative abilities with regards to cancer were evaluated with area under the curve (AUC) for receiver operating characteristic (ROC) curves and logistic regression analyses, calculating sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for adjusted models. The Youden's index was used as cutoff value.[34]

SAS Enterprise Guide 7.11 (SAS Institute, Cary, NC) and R 3.2.2 (The R Foundation, Vienna Austria) were used for statistical analysis. Figures were created with GraphPad Prism 6.07 (GraphPad Software, Inc., La Jolla, CA).

A p-value <0.05 was considered to be statistically significant.

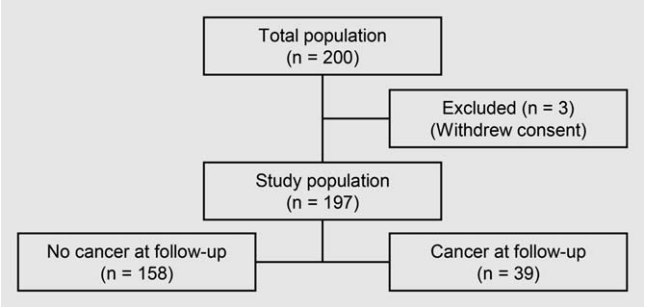

Figure 1. Flow-chart for the study population.

Ethical considerations

All patients received oral and written information and gave written consent before inclusion. The study was approved by the Scientific Ethics Committee of The Capital Region of Denmark (protocol number H-4–2013-063) and complied with the Declaration of Helsinki.

Results

Two hundred patients were included at their first visit to the DOC, 3 patients withdrew consent and the final study population included 197 patients (Fig. 1). Of these, 39 patients (19.8%) were diagnosed with the following malignant diseases: 11 patients were diagnosed with lung cancer, 8 with colorectal cancer, 4 with prostate cancer, 2 with breast cancer, 2 with B-cell lymphoma and 12 with other malignant diagnoses.

During 12-month follow-up, none of the remaining 158 patients were subsequently diagnosed with cancer; 57 of these patients had no abnormalities detected, while 101 were diagnosed with other nonmalignant diseases, including infections ($n = 9$), endocrinologic- ($n = 8$), gastrointestinal- ($n = 28$), cardiovascular- ($n = 6$), hepatologic- ($n = 8$), pulmonary- ($n = 7$), inflammatory- ($n = 12$) and other disorders ($n = 23$).

Patient characteristics are presented in Table 1.

Patients in the group diagnosed with cancer were significantly older and had a higher burden of comorbidities and previous cancer diagnoses compared to the group with no cancer at follow-up (Table 1). A total of 135 patients reported a weight loss at their first consultation, and out of these, 25 (18.5%, $p = 0.53$) were diagnosed with cancer. Mean self-reported weight loss was not significantly different between the groups. Among standard biomarkers, albumin and hemoglobin were lower among the cancer patients, while CRP and ESR were elevated compared to patients without

Table 1. Patient characteristics for the total patient population, patients without a final diagnosis of cancer and patients with a final diagnosis of cancer

		No cancer				*p* Values		
	Total	All	No abnormality detected	Nonmalignant disease	Cancer	*vs.* All	*vs.* NAD	*vs.* NMD
n (%)	197 (100)	158 (80.2)	57 (28.9)	101 (51.3)	39 (19.8)			
Sex, *n* (%)								
Female	101 (51.3)	85 (84.2)	34 (33.6)	51 (50.5)	16 (15.8)			
Male	96 (48.7)	73 (76.0)	23 (24.0)	50 (52.1)	23 (24.0)	0.21	0.10	0.35
Age, mean ± SD	62.7 ± 14.0	60.9 ± 14.4	57.0 ± 15.3	63.2 ± 13.4	69.7 ± 9.9	<0.0001	<0.0001	0.002
Weight loss (kg), mean ± SD	6.8 ± 6.0 (*n* = 177)	6.7 ± 6.0	6.7 ± 6.0 (*n* = 52)	6.7 ± 6.0 (*n* = 94)	7.4 ± 6.1 (*n* =31)	0.58	0.63	0.60
Previous cancer, *n* (%)								
No	181 (91.9)	149 (82.3)	53 (29.3)	96 (53.0)	32 (17.7)			
Yes	16 (8.1)	9 (56.3)	4 (25.0)	5 (31.3)	7 (43.8)	0.02	0.11	0.04
Charlson score, *n* (%)								
Charlson score 0–1	157 (79.7)	131 (83.4)	50 (31.9)	81 (51.6)	26 (16.6)			
Charlson score >1	40 (20.3)	27 (67.5)	7 (17.5)	20 (50.0)	13 (32.5)	0.04	0.02	0.12
Biochemistry, median (IQR)								
Standard biomarkers								
Albumin (g/L)	38 (34–41) (*n* = 196)	38 (34–41)	40 (35–42) (*n* = 56)	38 (34–41)	35 (31-38) (*n* = 39)	0.003	0.0005	0.02
CRP (mg/L)	3 (1–11) (*n* = 179)	2 (1–7)	1 (1–4) (*n* = 47)	3 (1–9) (*n* = 95)	11 (6-39) (*n* = 37)	<0.0001	<0.0001	<0.0001
Hemoglobin (mmol/L)	8.3 (7.5–8.9)	8.4 (7.8–9.0)	8.5 (7.8–9.1)	8.4 (7.8–8.9)	7.5 (6.5-8.6)	0.001	0.002	0.005
LDH (IU/L)	164 (145–196) (*n* = 195)	162 (146–194)	157 (142–173) (*n* = 56)	167 (148–197) (*n* = 100)	173 (143-211)	0.25	0.08	0.53
WBC count (10^9/L)	7.7 (6.4–9.0)	7.6 (6.2–8.8)	7.5 (6.1–9.0)	7.6 (6.6–8.8)	8.3 (6.5-9.4)	0.05	0.05	0.11
ESR (mm)	12 (5–23) (*n* = 191)	9 (5–20)	7 (3–13) (*n* = 56)	12 (5–22) (*n* = 98)	23 (16-39) (*n* = 37)	<0.0001	<0.0001	0.001
Immunological biomarkers								
suPAR (ng/mL)	3.1 (2.3–4.6) (*n* = 190)	2.9 (2.2–4.2)	2.5 (2.0–3.1) (*n* = 55)	3.5 (2.5–4.6) (*n* = 97)	4.7 (3.1-6.8) (*n* = 38)	<0.0001	<0.0001	0.003
Ficolin-1 (ng/mL)	191.8 (80.4–295.9) (*n* = 185)	195.0 (81.3–287.6)	132.5 (60.0–270.9) (*n* = 53)	197.1 (87.3–287.6) (*n* = 93)	179.0 (75.4-411.0)	0.62	0.22	0.98
Ficolin-2 (μg/mL)	5.0 (3.1–7.4) (*n* = 184)	4.8 (2.9–7.0)	5.3 (3.5–7.3) (*n* = 53)	4.3 (2.6–6.9) (*n* = 92)	5.2 (3.8-8.2)	0.20	0.78	0.09
Ficolin-3 (μg/mL)	24.7 (10.8–40.0) (*n* = 185)	25.0 (11.7–40.2)	24.2 (11.5–34.8) (*n* = 53)	28.2 (12.6–44.0) (*n* = 93)	21.8 (7.9-34.5)	0.31	0.83	0.17
MBL (μg/mL)	1.99 (0.79–5.78) (*n* = 185)	2.08 (0.83–5.88)	2.08 (1.15–5.49) (*n* = 53)	2.07 (0.72–6.31) (*n* = 93)	1.89 (0.57-5.70)	0.59	0.60	0.65
Pentraxin-3 (ng/mL)	2.9 (1.5–5.2) (*n* = 185)	2.8 (1.5–5.1)	1.9 (1.5–4.2) (*n* = 53)	2.9 (1.5–5.2) (*n* = 93)	3.9 (2.7-6.1)	0.05	0.01	0.18

n is added in parentheses for variables with missing values.
Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; LDH, lactate dehydrogenase; MBL, mannose-binding lectin; NAD, no abnormality detected; NMD, nonmalignant disease; SD, standard deviation; suPAR, soluble urokinase plasminogen activator receptor; WBC, white blood cell.

Table 2. Associations between potential risk factors and cancer

	Univariate		Adjusted for age and sex		Adjusted for age, sex and CRP	
Variable	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
Sex (male)	1.67 (0.82–3.41)	0.16	1.83 (0.87–3.82)	0.11	1.57 (0.70–3.53)	0.27
Age (per year)	1.05 (1.02–1.08)	0.0007	1.05 (1.02–1.09)	0.0006	1.03 (1.00–1.07)	0.06
Weight loss (per 5 kg)	0.92 (0.63–1.33)	0.65	0.90 (0.62–1.30)	0.57	0.92 (0.60–1.39)	0.69
Previous cancer	3.62 (1.26–10.44)	0.02	3.57 (1.14–11.23)	0.03	9.27 (2.20–39.01)	0.002
Charlson score	1.49 (1.08–2.05)	0.01	1.29 (0.93–1.81)	0.13	1.26 (0.88–1.78)	0.20
Albumin (log2)	0.92 (0.41–2.03)	0.83	1.46 (0.50–4.27)	0.49	2.60 (0.77–8.76)	0.12
LDH (log2)	2.04 (1.05–3.98)	0.04	1.71 (0.86–3.40)	0.13	1.46 (0.73–2.95)	0.29
Hemoglobin (log1.1)	0.64 (0.51–0.81)	0.0003	0.70 (0.54–0.90)	0.006	0.82 (0.62–1.08)	0.15
White blood cell count	1.18 (1.03–1.36)	0.02	1.18 (1.01–1.36)	0.03	1.11 (0.97–1.28)	0.14
CRP (log2)	1.50 (1.26–1.79)	<0.0001	1.41 (1.18–1.70)	0.0002	1.41 (1.18–1.70)	0.0002
ESR (log2)	1.62 (1.26–2.09)	0.0002	1.44 (1.10–1.89)	0.007	1.06 (0.76–1.46)	0.75
suPAR (log2)	3.36 (1.91–5.93)	<0.0001	2.58 (1.41–4.71)	0.002	2.33 (1.19–4.58)	0.01
Ficolin-1 (log2)	1.04 (0.82–1.32)	0.74	1.00 (0.77–1.28)	0.97	0.84 (0.64–1.11)	0.22
Ficolin-2 (log2)	1.34 (0.88–2.03)	0.17	1.40 (0.91–2.16)	0.13	1.25 (0.78–2.02)	0.36
Ficolin-3 (log2)	0.92 (0.71–1.17)	0.48	0.95 (0.73–1.23)	0.69	0.90 (0.67–1.19)	0.45
MBL (log2)	0.95 (0.77–1.16)	0.60	0.98 (0.79–1.21)	0.84	0.95 (0.75–1.19)	0.63
Pentraxin-3 (log2)	1.18 (0.89–1.55)	0.24	1.16 (0.85–1.58)	0.36	0.99 (0.68–1.43)	0.94

Abbreviations: CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; MBL, mannose-binding lectin; suPAR, soluble urokinase plasminogen activator receptor.

cancer, patients with no abnormalities detected as well as patients with other nonmalignant diseases (Table 1). There was a borderline significant increase in white blood cell count among cancer patients (Table 1).

Patients with cancer had significantly higher suPAR and there was a borderline significant increase in pentraxin-3 compared to cancer-free patients, but none of the other PRRs were significantly different between the two groups (Table 1). The group of patients who had no abnormalities detected had markedly lower levels of CRP, ESR, suPAR and pentraxin-3, whereas albumin and hemoglobin was higher compared to patients diagnosed with cancer (Table 1). Patients diagnosed with other nonmalignant diseases had biomarker levels between that of cancer patients and patients with no abnormalities detected (Table 1).

Compared to the patients who had no abnormalities detected, the 12 patients with inflammatory disorders had significantly lower median (IQR) albumin [32 g/L (30–38), $p = 0.004$] and hemoglobin [7.8 mmol/L (7.0–8.4), $p = 0.03$] levels, and significantly higher median (IQR) CRP [11.5 mg/L (4.0–51.5), $p < 0.001$], ESR [21 mm (10–67), $p = 0.0008$] and suPAR [4.2 ng/mL (3.4–5.3), $p = 0.008$]. In addition, there was a trend toward increased ficolin-1 [244.7 ng/mL (146.9–454.4), $p = 0.06$] and pentraxin-3 [5.1 ng/mL (2.8–6.8), $p = 0.07$] levels in these patients.

In univariate logistic regression analyses, age, previous cancer, Charlson score, lactate dehydrogenase, hemoglobin, white blood cell count, CRP, ESR and suPAR were significantly associated with newly diagnosed cancer during follow-up (Table 2).

When adjusted for both age and sex, the following remained significantly associated with newly diagnosed cancer during follow-up: age, previous cancer, hemoglobin, white blood cell count, CRP, ESR and suPAR (Table 2). None of the soluble PRRs investigated were significantly associated with cancer diagnoses (Table 2).

When CRP was included in the adjusted analysis along with age and sex, only previous cancer, suPAR and CRP itself remained significantly associated with newly diagnosed cancer (Table 2).

To investigate the relationship between the three inflammatory biomarkers CRP, ESR and suPAR, we performed pairwise correlation analyses (Fig. 2). There was a strong positive correlation between CRP and ESR, and suPAR was also positively correlated with both CRP and ESR but to a lesser degree.

For the variables that remained significantly associated with incident cancer after adjustment for age and sex, we performed ROC curve analyses which resulted in the following AUCs (95% CI): age 0.675 (0.592–0.757), previous cancer 0.561 (0.498–0.625), hemoglobin 0.670 (0.565–0.775), white blood cell count 0.600 (0.496–0.704), CRP 0.761 (0.676–0.845), ESR 0.719 (0.627–0.810) and suPAR 0.721 (0.632–0.810).

Furthermore, to compare the predictive values of CRP and suPAR, we performed ROC curve and logistic regression

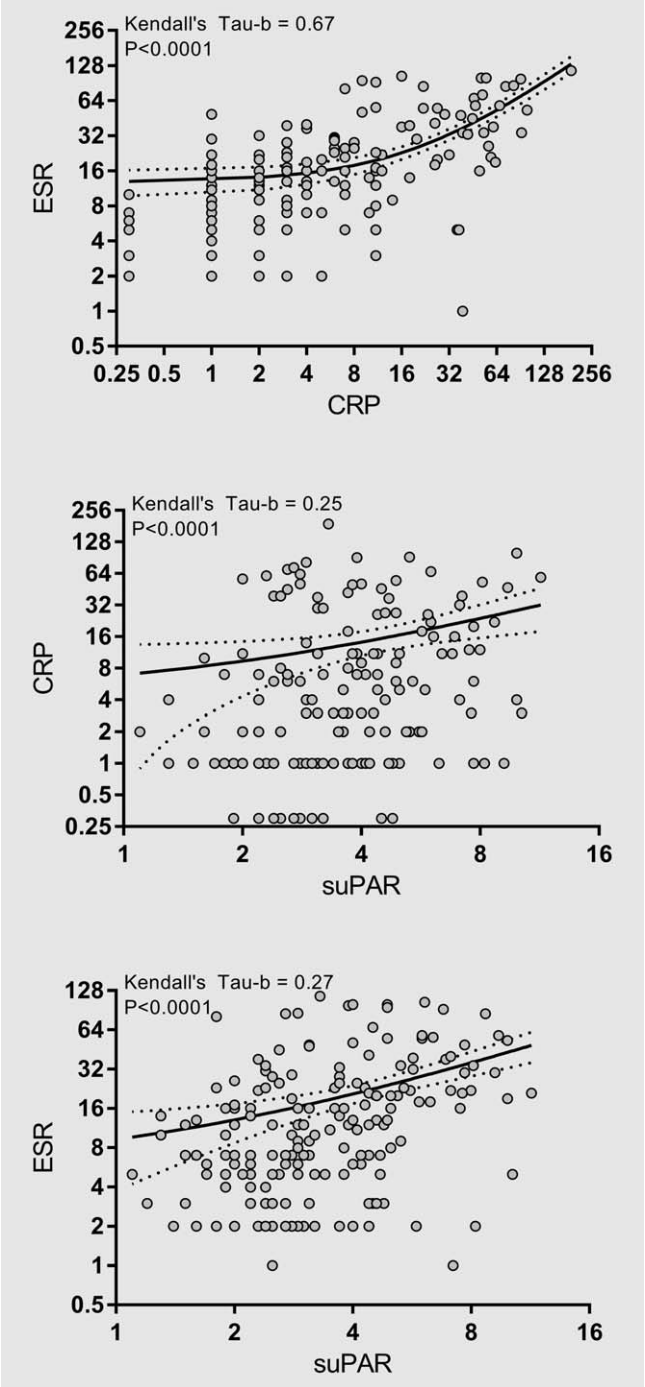

Figure 2. Pairwise correlations between C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and soluble urokinase plasminogen activator receptor (suPAR) with Kendall's Tau-b correlation coefficient and linear regression line. Note the logarithmic axes.

analyses for a full model containing age, sex, previous cancer, CRP and suPAR and compared it with a model without CRP and a model without suPAR, respectively (Table 3). Combining both CRP and suPAR gave the highest AUC, sensitivity and an NPV of 93.4% (Table 3).

Discussion

In the present study, we evaluated known and novel biomarkers for their association with cancer. We found that hemoglobin, white blood cell count, CRP, ESR and the biomarker suPAR as well as previous cancer were associated with incident cancer after adjustment for both age and sex. When CRP was included in the adjusted analyses, only previous cancer, CRP and suPAR remained significantly associated with cancer; patients with previous cancer had the highest risk, and the AUC, sensitivity and NPV were improved when using CRP and suPAR together in the full model.

Fast diagnosis and subsequent initiation of correct treatment is vital for the overall prognosis in patients diagnosed with cancer.[35–37] Prolonging the diagnostic time interval increases the risk of a more advanced stage of the cancer at the time of diagnosis,[37] and survival is decreased with increasing stage of the disease at diagnosis.[36] Therefore, it is equally important to successfully and rapidly detect and diagnose cancers and not end the diagnostic process prematurely in patients who suffer from unrecognized serious diseases.

NSSC patients in fast-track DOCs pose a diagnostic challenge as they represent a heterogeneous group that suffer from a wide range of conditions, ranging from various cancers to infections, autoimmune diseases and healthy individuals.[2] Developing a diagnostic strategy that covers a broad variety of conditions is thus needed. A variety of biomarkers might help in screening and early detection of cancer[6] but many have not been evaluated in clinical use.

Although the biomarkers that were significantly associated with incident cancer trended toward abnormal values in patients with cancer, the median levels were virtually within the normal reference ranges, complicating clinical decision making. However, patients with no abnormalities detected had low CRP and suPAR levels—which were comparable to that of healthy people in the general population, including blood donors[38–40]—while patients receiving a diagnosis had increased CRP and suPAR levels, especially patients with incident cancer. Both CRP and suPAR contributed to an improved negative predictive value.

CRP, ESR and the novel inflammatory biomarker suPAR were all positively correlated with each other, with the strongest correlation found between CRP and ESR, consistent with previous research.[41] suPAR showed a weaker correlation with CRP and ESR, possibly because the biomarkers reflect different aspects of inflammation,[42] but, more likely, the difference between suPAR and the other inflammatory biomarkers could be due to the high expression of uPAR on cancer cells and surrounding stromal cells,[18–20] causing the increase in suPAR levels.

To our knowledge, this is the first study investigating the association between MBL, ficolin-1–3 and pentraxin-3 with incident cancer in a group of NSSC patients. Several studies have previously found an association between increased levels of these biomarkers and various organ-specific cancers.[7,8,10,12–15] For

Table 3. Logistic regression and ROC curve analysis

	AUC (95% CI)	*p*	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
Age, sex, previous cancer, CRP, suPAR	0.802 (0.723–0.881)	0.02[1]	0.806 (0.676–0.935)	0.728 (0.653–0.803)	0.934 (0.887–0.981)	0.439 (0.320–0.559)
Age, sex, previous cancer, CRP	0.759 (0.669–0.848)	0.07[2]	0.722 (0.576–0.869)	0.743 (0.669–0.816)	0.910 (0.857–0.963)	0.426 (0.302–0.550)
Age, sex, previous cancer, suPAR	0.776 (0.691–0.862)	0.13[2]	0.694 (0.544–0.845)	0.787 (0.718–0.856)	0.907 (0.854–0.959)	0.463 (0.330–0.596)

[1]Comparison of all three ROC curves, 2 degrees of freedom.
[2]Pairwise difference, compared to the full model, 1 degree of freedom.
Abbreviations: AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; NPV, negative predictive value; PPV, positive predictive value; suPAR, soluble urokinase plasminogen activator receptor.

example, pentraxin-3 levels have been found to be associated with lung cancer,[7] gliomas[8,9] and prostate cancer,[10] and high pentraxin-3 expression was found to be associated with poor survival in lung cancer.[7,43] Similarly, ficolin-1 (M-ficolin) has been associated with colorectal cancer,[12] and serum concentrations of ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin) have been found to be increased in patients with malignant ovarian tumors compared to benign tumors or normal ovaries.[13,14] Also, MBL concentration has been found to be increased in patients with colorectal cancer,[15] advanced ovarian cancer,[16] glioblastoma multiforme[17] and thyroid cancer.[11]

On the contrary, serum and intrahepatic ficolin-2 levels have also been found to be decreased in patients with hepatocellular carcinoma compared to healthy persons,[44] and in children with cancer, serum ficolin-1 levels were not different from age-matched controls.[45] In our patient cohort, these PRRs did not add diagnostic information; pentraxin-3 was significantly higher in patients with cancer compared to patients with no abnormalities detected, but this association was not significant in the adjusted analyses. Even though these biomarkers are not relevant in cancer diagnostics for NSSC patients, they could still be useful in selected cancer cohorts. For the patients with inflammatory disorders, we observed a trend toward increased ficolin-1 and pentraxin-3 compared to patients with no abnormalities detected, suggesting that these PRRs are associated with inflammatory disease. This is supported by a systematic review and meta-analysis of 20 studies, where serum and plasma levels of pentraxin-3 were found to be elevated in autoimmune and inflammatory disorders compared to normal controls.[46] Similarly, plasma ficolin-1 has been found to be associated with rheumatoid arthritis,[47] but no significant difference was observed in ficolin-1 levels between systemic lupus erythematosus patients and healthy controls.[48]

Regarding the medical history factors examined, the Charlson comorbidity score showed a moderate association with cancer in NSSC patients, but this disappeared in the multivariate models.

Conflicting results exist concerning an association between involuntary weight loss and cancer for patients with nonspecific symptoms.[49,50] Interestingly, involuntary weight loss is often the major cause of concern of cancer and referral to the DOC, but it was not associated with incident cancer in our study. As weight loss in this study was self-reported it may be subject to recall bias. Similar to previous findings, cancer was only diagnosed in 18.5% of the patients who reported a weight loss.[1]

As a fast-track cancer pathway for NSSC patients, the purpose of the DOCs is to uncover hidden malignancy as well as exclude other present serious disease with a high degree of certainty. Discovery of a single or a collection of biomarkers that can rule out cancer with high specificity would be an invaluable addition to both cancer screening in the general population as well as in evaluation of patients with NSSC. The biomarker suPAR was shown to add significant and independent value to diagnosing cancer among patients with NSSC. Thus, combining suPAR with other independent factors may improve diagnostic efficacy. Interestingly, suPAR has previously been shown to carry prognostic value in cancer patients[27,51] and may as such add to both diagnosing and prognosticating cancer patients and accordingly uPAR is currently pursued as a promising imaging and radiotherapeutic target.[52–55] In any case, the positive and negative predictive values must be examined in a larger patient cohort to establish a clinical cutoff, and combining high sensitivity biomarkers with high specificity biomarkers to detect cancer[6] or constructing a predictive model consisting of biomarkers and clinical information would also require development and validation in cohorts larger than the present.

Strength and limitations

The examined cohort is small with few cases of cancers and therefore has character of a pilot study. As mentioned, weight loss was self-reported and may be subject to recall bias. Furthermore, the patients have a high degree of comorbidity and competing illnesses which makes the data more difficult to interpret, but this also strengthens the study as this patient sample is highly representative of the entire population of NSSC patients, making the results from this prospective study applicable in clinical practice. A larger patient cohort is also

needed to establish how these biomarkers relate to other serious nonmalignant diagnoses.

Conclusion

In this study, previous cancer, CRP and suPAR were significantly associated with cancer diagnosis in patients with nonspecific signs and symptoms. The PRRs ficolin-1–3, MBL and pentraxin-3 were not associated with cancer.

To examine the optimal screening strategy using biomarkers to identify cancer, more studies are required. The inflammatory biomarker suPAR is a promising new biomarker in the DOC setting, but the predictive values of suPAR in patients with NSSC should be examined in a larger patient material.

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