alone, its use as a negative prognostic indicator has been well documented [58–60].

In the current study, the combined evaluation of a broad array of cancer-associated serum proteins along with several distinct bioinformatic approaches has led to the identification of a multimarker panel which demonstrates proof-of-principle for the development of biomarker based diagnostic tools for NSCLC. The diagnostic capability of the approach described herein meets or exceeds several recent reports regarding the use of biomarker panels in lung cancer diagnosis in terms of SN, SP, and accuracy, and the relatively small size of the optimal panel of MIF, PRL and THSP coupled with the overall flexibility of our classification algorithm make the current strategy an attractive alternative for clinical development [27,61,62]. The MMC algorithm has also been successfully applied in two recent studies regarding the diagnosis of ovarian cancer [30,63]. The performance demonstrated by our selected panel in the current study does not reach the level recommended by the FDA for a successful diagnostic test for lung cancer. Thus, this panel would not serve as an effective standalone test. However, a second-line test utilizing these biomarkers, utilized as an adjunct to CT-scanning, may prove effective. In this setting, a diagnostic biomarker panel must perform at a maximal level of SN to ensure that all cases are promptly referred for pathologic examination while maintaining a suitable level of SP in order to reduce the number of false positive test results generated by CT. The MIF, PRL, THSP panel indentified here provided levels of SP ranging from 30–56% at SNs greater than 90%, illustrating its potential. Further evaluation of this panel should be designed to examine its ability to classify highly suspicious CT-identified nodules as cancer or benign. Although the consistent selection of PRL and THSP by each of our evaluated algorithms represents a significant step forward in the development of diagnostic protein signatures, the identification of additional useful biomarkers will be required to achieve a performance worthy of clinical development. Upon further validation and optimization, serum biomarker panels could provide an effective means of further assessing the malignant potential of patients designated as having a high risk for lung cancer on the basis of CT findings.

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Supplemental material

Table S1
Intra-assay reproducibility of evaluated biomarkers

Biomarker	%CV	Biomarker	%CV
ACTH	3.5	M-CSF	4.1
Adiponectin	2.3	Mesothelin	3.8
AFP	1.8	MICA	4.1
Angiostatin	4	MIF	7
bFGF	4.9	MMP-1	3
CA 15-3	4	MMP-12	3.3
CA 19-9	4.4	MMP-7	5.6
CA 72-4	2.9	MMP-8	3.3
CD40L (TRAP)	7.7	MMP-9	1.4
CEA	5.6	MPO	5.6
Cyfra 21-1	3.7	NGF	5.1
DR5	4	PDGF-BB	2.7
EGF	4.3	Prolactin	4.9
EGFR	2.6	RANTES	2.7
Eotaxin-1	5.1	Resistin	6.2
ErbB2	2.8	SAA	7.4
FSH	3.2	SCC	3.4
GCSF	3.01	SCF	5.2
GH	2.8	SCGF-B	3
GROα	6.8	SDF-1α	3.2
HE4	4.7	sE-Selectin	9.9
HGF	4	sFas	4.2
HSP 70	3.1	sFasL	5.5
IGFBP-1	5.5	sI-CAM	3.2
IL-1Rα	5.4	sV-CAM	2.4
IL-2R	4.1	Thrombospondin	2.9
IL-6	5.8	TNF-RI	5.3
IL-6R	1.3	TNF-RII	2.9
IL-8	3.2	TNF-α	3.9
IP-10	4.2	tPAI-1	3.9
Kallikrein 10	2.4	TRAIL	3.1
Leptin	3.6	TSH	3
LH	2.6	TTR	3.1
LIF	4.3	ULBP-1	11.4
MCP-1	6.9	ULBP-2	4.6
MCP-3	6.2	VEGF	4.9

%CV based on duplicate fluorescence measurements of each experimental sample.

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