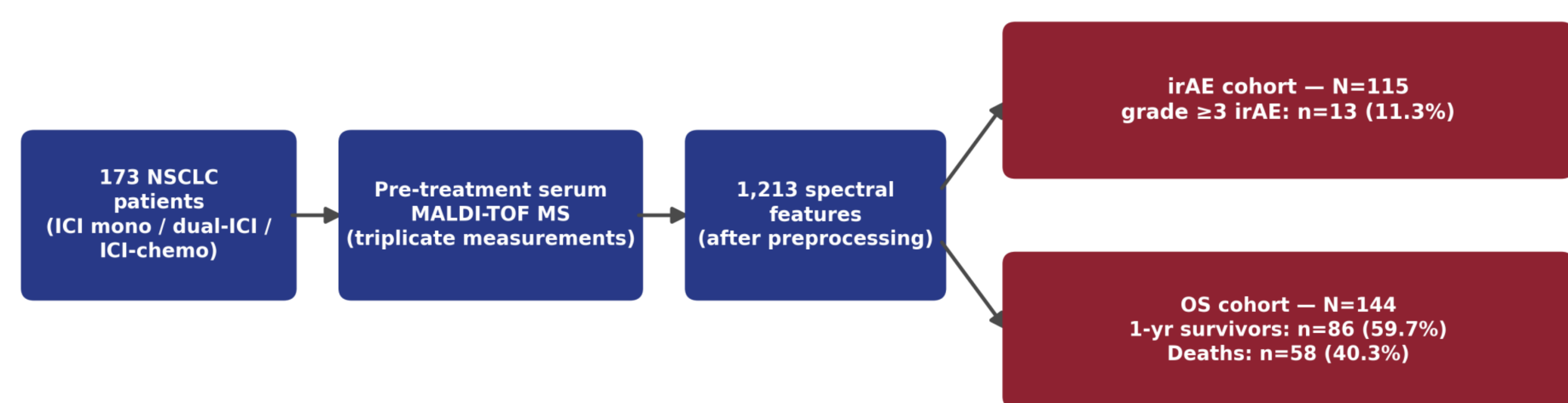


INTRODUCTION

- Severe immune-related adverse events (irAEs; grade ≥ 3) occur in approximately 15% of patients with NSCLC receiving immune checkpoint inhibitors (ICIs) and can result in treatment discontinuation, hospitalization, and substantial morbidity.
- Current biomarkers, including PD-L1 expression and tumor mutational burden (TMB), provide limited ability to predict severe toxicity or survival outcomes at the individual-patient level.
- Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry enables rapid, low-cost serum proteomic profiling from a single pre-treatment blood sample.
- We evaluated whether baseline MALDI-TOF proteomic signatures combined with machine-learning algorithms could predict grade ≥ 3 irAEs and 1-year overall survival (OS) before initiation of immunotherapy.

METHODS



- Study design:** Retrospective cohort study of 173 patients with NSCLC treated with immune checkpoint inhibitor (ICI) monotherapy, dual-ICI therapy, or ICI plus chemotherapy.
- Proteomic profiling:** Pre-treatment serum samples were analyzed using MALDI-TOF mass spectrometry with triplicate measurements. Following preprocessing, 1,213 proteomic spectral features were included for analysis.
- Study cohorts:**
 - irAE cohort:** 115 patients with complete toxicity documentation, including 13 patients (11.3%) who experienced grade ≥ 3 immune-related adverse events (irAEs).
 - Overall survival (OS) cohort:** 144 patients with definitive 1-year survival outcomes, including 86 survivors (59.7%) and 58 confirmed deaths (40.3%).
- Machine-learning model:** XGBoost classifiers were developed to predict grade ≥ 3 irAEs and 1-year OS. Hyperparameters were optimized using Bayesian optimization (Optuna; 100 trials).
- Model validation:** Performance was evaluated using out-of-bag bootstrap validation (100 iterations). For the irAE model, significance was further assessed using permutation testing (1,000 iterations).

DISCUSSION AND CONCLUSION

Actionable Take Away Message

- Novel proteomic toxicity biomarker:** To our knowledge, this represents the first MALDI-TOF-based classifier developed specifically to predict grade ≥ 3 immune-related adverse events (irAEs) in NSCLC patients receiving immunotherapy.
- Practical clinical workflow:** MALDI-TOF serum profiling can be performed from a single pre-treatment blood sample with rapid turnaround and lower cost than many high-dimensional proteomic platforms.
- Dual predictive capability:** Baseline proteomic signatures were associated with both severe irAE risk and 1-year survival, potentially enabling simultaneous assessment of treatment risk and expected benefit before therapy initiation.
- Potential clinical value:** Identification of patients at elevated toxicity risk could support intensified monitoring, earlier subspecialty involvement, and more informed treatment discussions.

Future Direction for Research

- External validation:** Prospective multicenter studies are needed to confirm model performance across institutions, patient populations, and immunotherapy regimens.
- Biological characterization:** Future LC-MS/MS or DeepMALDI analyses may identify the proteins underlying key discriminatory spectral features.
- Integrated biomarker development:** Combining MALDI-TOF proteomic signatures with PD-L1 expression, TMB, and systemic inflammatory markers may improve predictive performance.
- Clinical utility studies:** Prospective evaluation is needed to determine whether proteomic risk stratification meaningfully influences clinical decision-making and patient outcomes.

Limitations

- Retrospective, single-center study.
- Modest sample size, particularly for grade ≥ 3 irAEs (n=13 events).
- Lack of external validation cohort.
- Findings should be considered hypothesis-generating pending prospective validation.

Conclusions

- Pre-treatment MALDI-TOF proteomic profiling combined with machine learning identified patients at increased risk for severe irAEs and poorer 1-year survival following immunotherapy.**
- If prospectively validated, this approach could provide a scalable and clinically practical tool for pre-treatment risk stratification in NSCLC.**

REFERENCES & ACKNOWLEDGEMENTS

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Artificial Intelligence (AI) Use Disclaimer

Claude (Anthropic) was used to (1) refine the language of the manuscript and abstract, and (2) write code for statistical analysis. All statistical outputs generated with AI-assisted code were independently reviewed and verified by the study authors. AI did not contribute to study design, data interpretation, or scientific conclusions, which remain the sole responsibility of the authors.

RESULTS

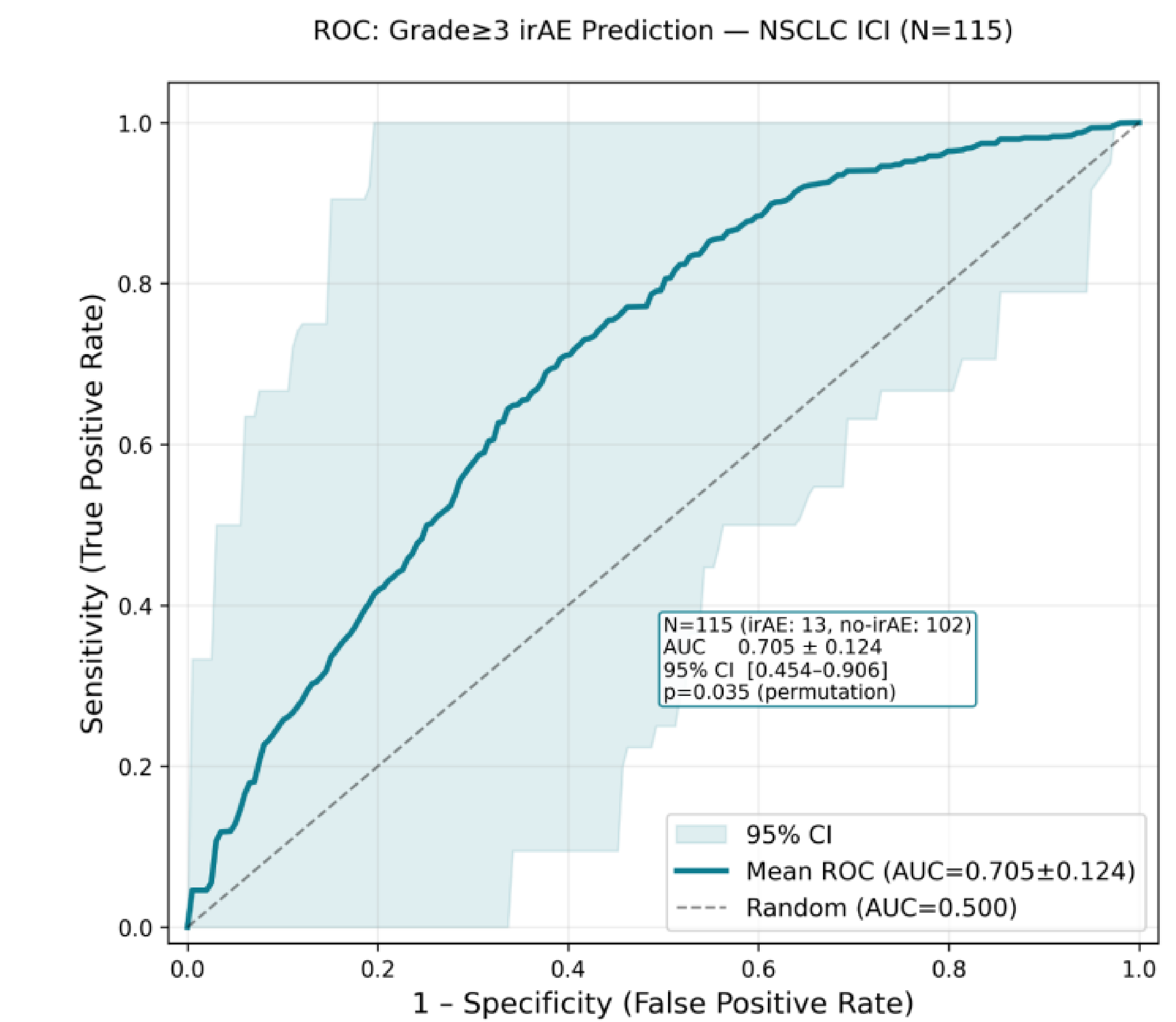


Figure 1: AUC curve for irAE prediction

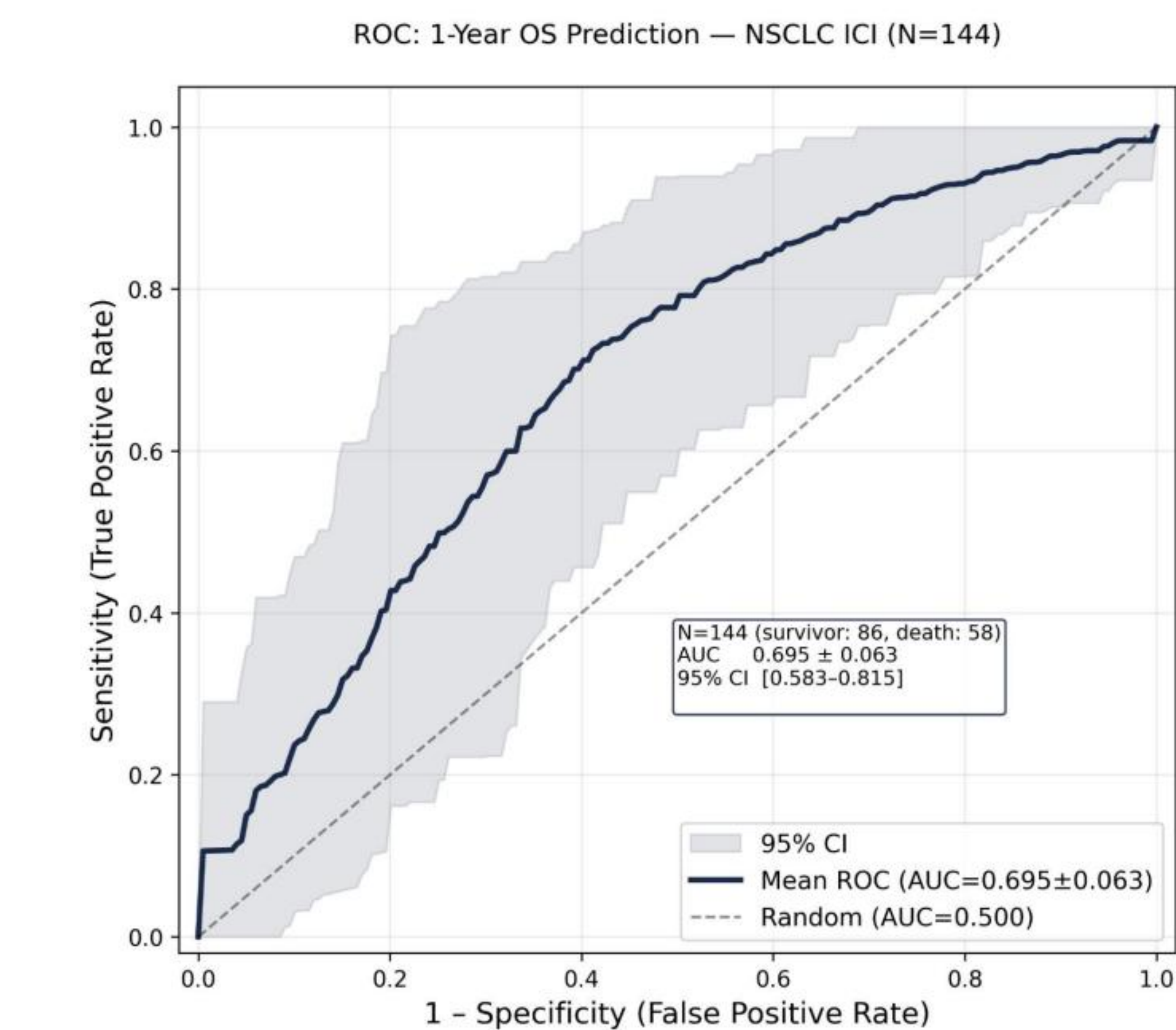


Figure 2: AUC curve for 1-year overall survival prediction