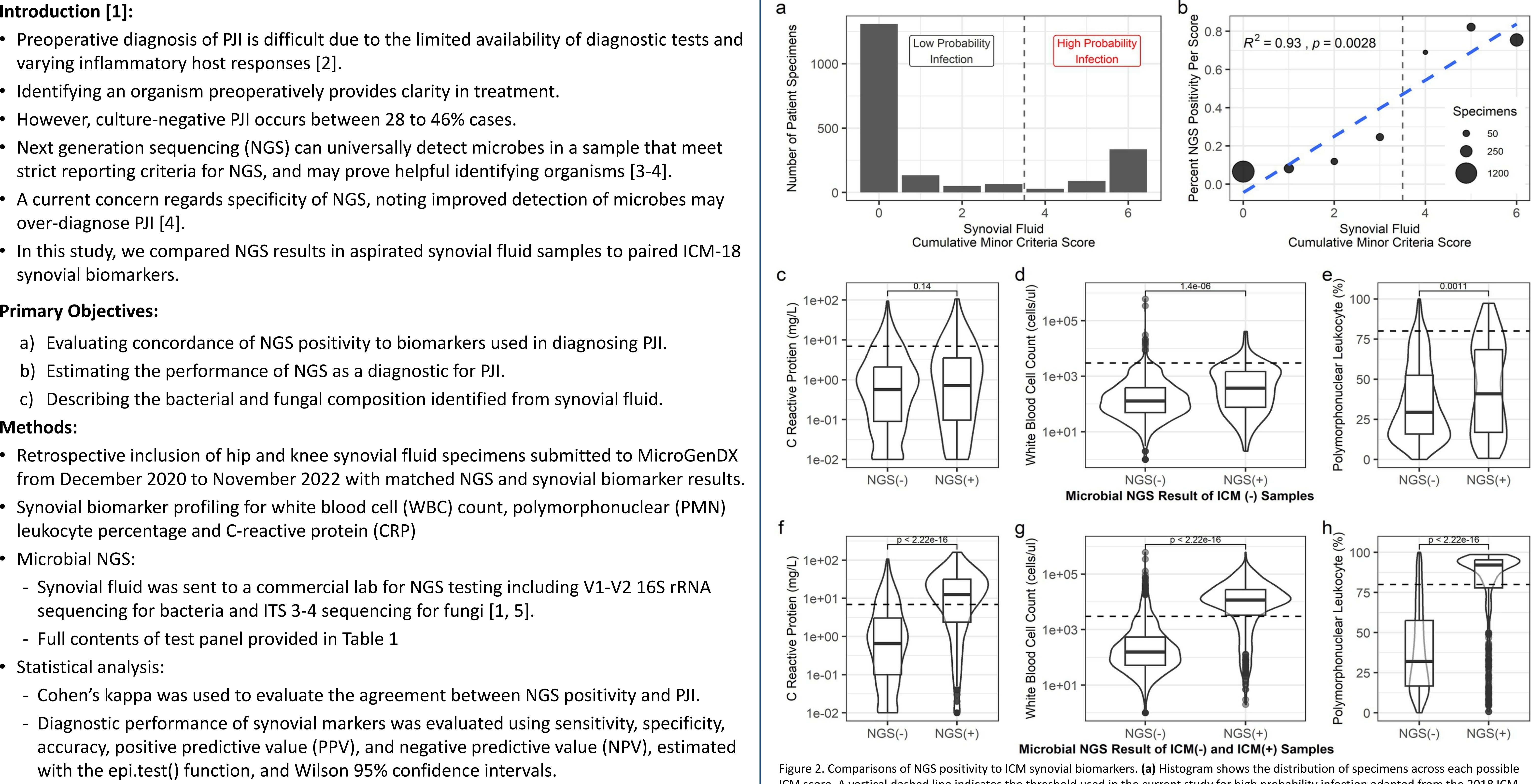


Microbial next generation DNA sequencing of aspirated synovial fluid shows concordance with ICM criteria biomarkers for diagnosing PJI

Craig D Tipton¹, Jacob Ancira^{1,2}, Saad Tarabichi³, Alisina Shahi⁴, Kayla Jarvis¹, Khalid Omeir^{1,2}, Nicholas Sanford¹, Nick A Tallman¹, Jennifer White¹, Caleb D Phillips², Javad Parvizi⁵, Edward J McPherson⁶

¹MicroGen DX, Lubbock, TX, United States, ²Department of Biological Sciences, Texas Tech University, Lubbock, TX, United States, ³Department of Orthopedic Surgery, Mayo Clinic Arizona, Phoenix, AZ, United States, ⁴Department of Orthopedic Surgery, University of Texas Health Science Center at Houston, Houston, TX, United States, ⁵International Joint Center, Acibadem University Hospital, Istanbul, Türkiye, ⁶Department of Orthopedic Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, United States



Test	Assay targets	Reports	Cutoff	ICM-18 score
qPCR panel	Universal 16S	Bacterial cell estimate		n/a
	Common species	Estimated count/μL		n/a
	Antimicrobial resistance	Presence/absence		n/a
Targeted NGS	V1-V2 16S rRNA	Bacterial profile (%)		n/a
	ITS3-4	Fungal profile (%)		n/a
Biomarkers	CRP	mg/L CRP	>6.9 mg/L	1
	WBC	Cells/μL WBC	>3000 cells/μL	3
	PMN%	% PMN	>80%	2

Results:

- 2,011 synovial fluid samples studied, the majority being knee samples (85.7%).
- 22.6% of samples were NGS-positive, predominantly for bacteria (448 vs. 26 fungal).
- Totals of 160 unique bacterial and fungal species observed, most abundant in Figure 1.
- Eight species were the dominant bacteria in 64% of cases, with remaining third made up of 61 less common species.
- Fungal detections generally rare, observed in 2.2% of all specimens led by *Candida*.
- 23.2% of samples met the minor criteria for high probability PJI (≥ 4 score; Figure 2a) and NGS positivity had substantial concordance with PJI (k=0.68).

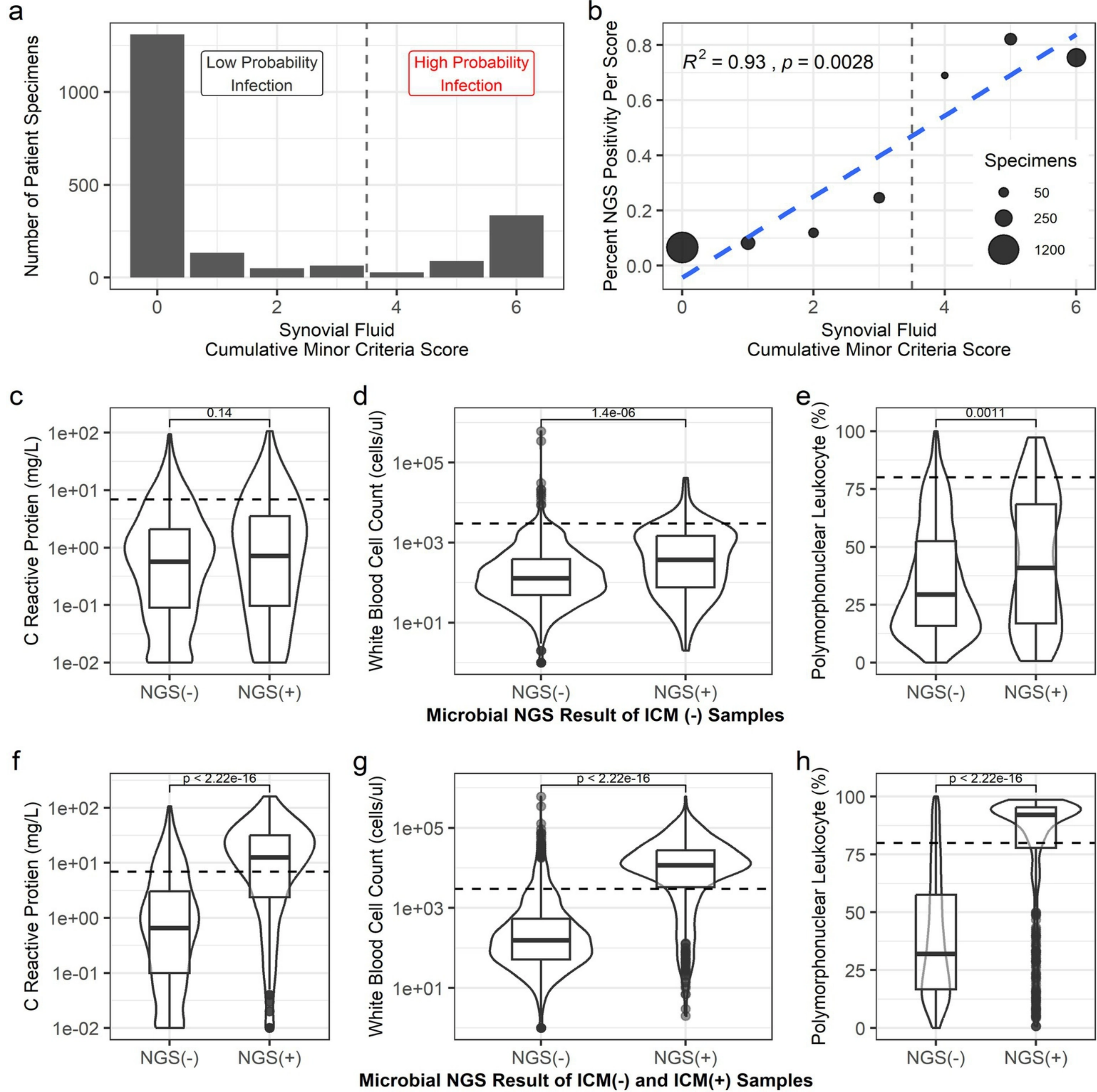
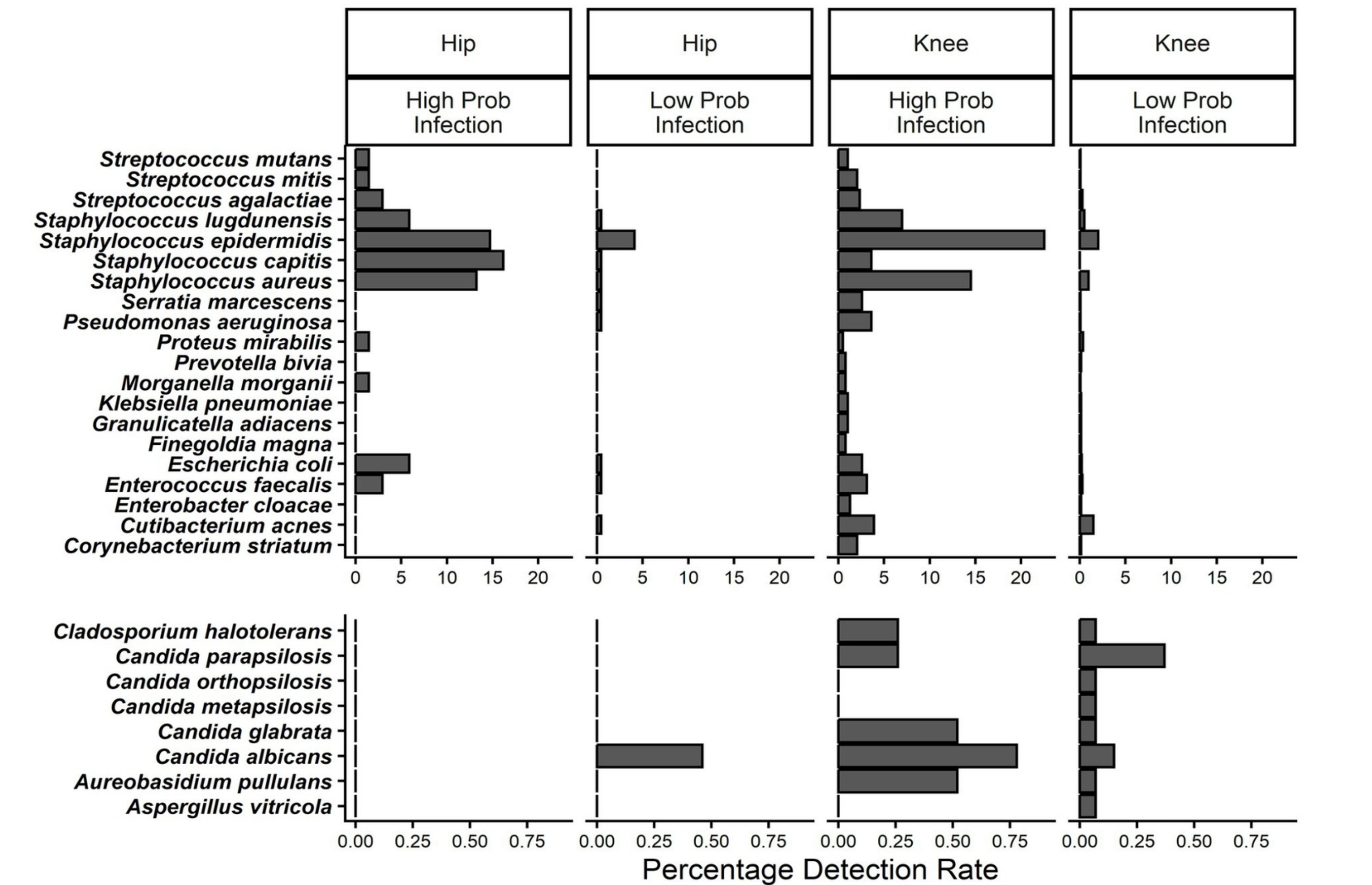


Figure 2. Comparisons of NGS positivity to ICM synovial biomarkers. (a) Histogram shows the distribution of specimens across each possible ICM score. A vertical dashed line indicates the threshold used in the current study for high probability infection adapted from the 2018 ICM minor criteria. (b) The percentage of NGS positive samples was calculated for each possible ICM score. A direct linear relationship was observed between cumulative ICM scores and aggregated NGS positivity. (c-h) Box plots show the relationship between NGS positivity and raw biomarker score distributions for (c,f) CRP, (d,g) WBC count, and (e,h) PMN%. For the first set (c-e), NGS positivity was compared to biomarkers in uninfected samples whereas (f-h) compares biomarker values in all samples by NGS positivity. In the box plots, box indicates the 1st and 3rd quartiles, the thicker banded line within the box indicates the median, and the whiskers indicate the bounds of the distribution. A violin plot layer visualizes the full data distribution. A two-tailed t-test was used to determine statistical significance by NGS positivity and each biomarker, with p-value shown in plot. A dashed horizontal line indicates ICM thresholds for elevated biomarkers.

Table 2: Comparison of diagnostic performance to ICM-18 minor criteria by synovial markers.

Synovial diagnostic	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	PPV (95% CI)	NPV (95% CI)
CRP	0.802 (0.763–0.836)	0.894 (0.878–0.908)	0.873 (0.858–0.887)	0.688 (0.647–0.726)	0.939 (0.926–0.95)
WBC	1 (0.992–1)	0.979 (0.97–0.985)	0.984 (0.977–0.988)	0.932 (0.906–0.951)	1 (0.997–1)
PMN (%)	0.936 (0.91–0.955)	0.947 (0.935–0.957)	0.945 (0.934–0.954)	0.838 (0.804–0.868)	0.981 (0.972–0.987)
NGS positivity	0.764 (0.723–0.801)	0.923 (0.909–0.935)	0.887 (0.873–0.9)	0.743 (0.702–0.781)	0.931 (0.917–0.942)

Key Result: NGS positivity correlates with synovial biomarkers

- NGS positivity correlated with cumulative biomarker score (R2=0.93, p=0.003, Figure 2b).
- Within ICM-negative samples, NGS positivity was significantly associated with both elevated WBC and PMN%, but not CRP (Figure 2c-e).
- Across all samples, NGS positivity was significantly associated with elevated results for all biomarkers tested (Figure 2f-h).
- The specificity of NGS was greater than CRP but less than WBC and PMN% (Table 2).

Discussion:

- Microbial targeted NGS positivity was concordant with ICM minor criteria for PJI
- NGS has a low false positive rate (92.3% specificity) comparable to established ICM biomarkers (89.4%–97.9%).
- NGS excels in detection of fastidious and atypical pathogens, whereas conventional testing may be unsuited for detection of up to a third of dominant pathogens.
- Presented findings suggest NGS offers broad discovery power for pathogens, is specific to PJI, and may warrant guidance as a diagnostic criterion when restructuring future diagnostic criteria defining PJI.

Key Takeaways:

- NGS correlates with clinical biomarkers for diagnosing PJI.
- NGS improves microorganism identification in preoperative evaluations.
- NGS should be considered in future diagnostic classifications.

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