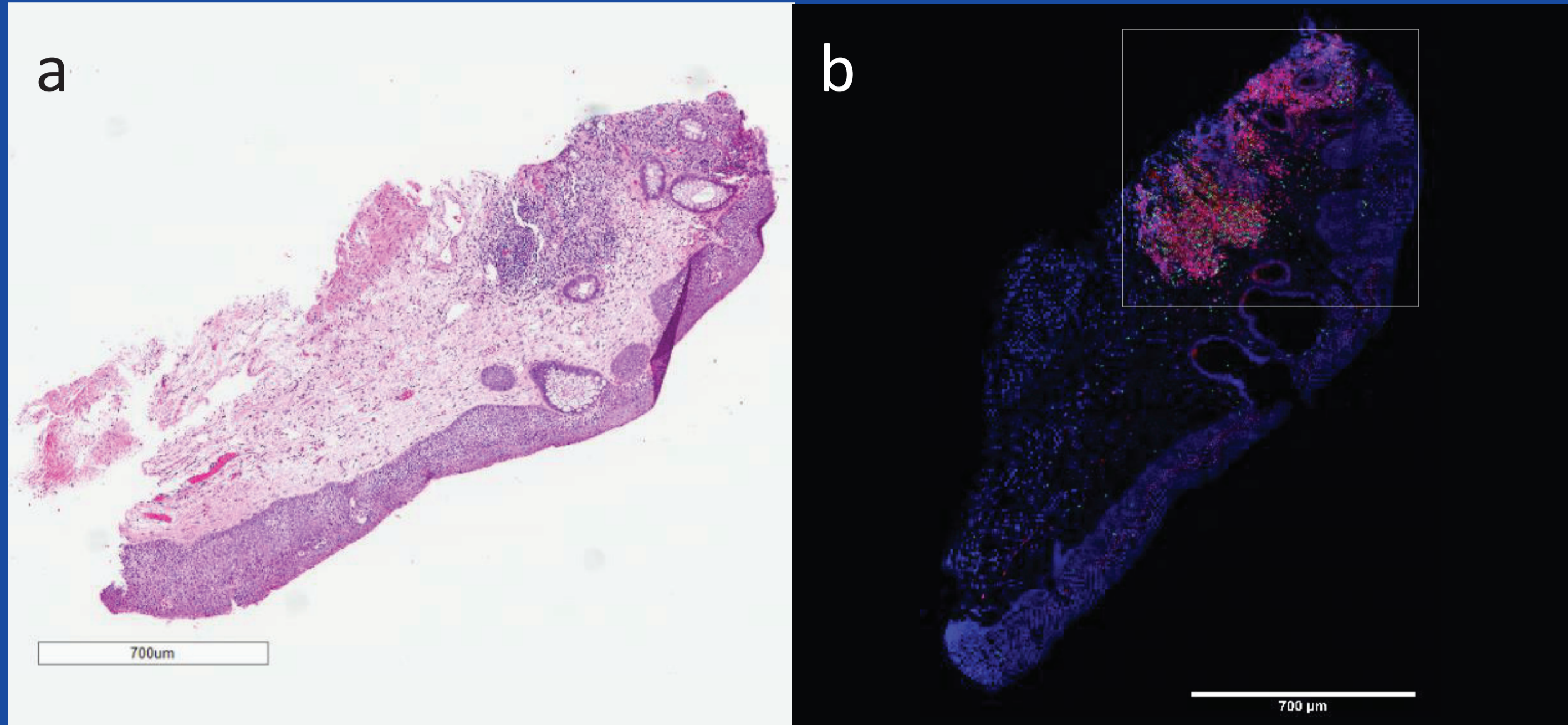


T-cells in the anal mucosa of men with HSIL

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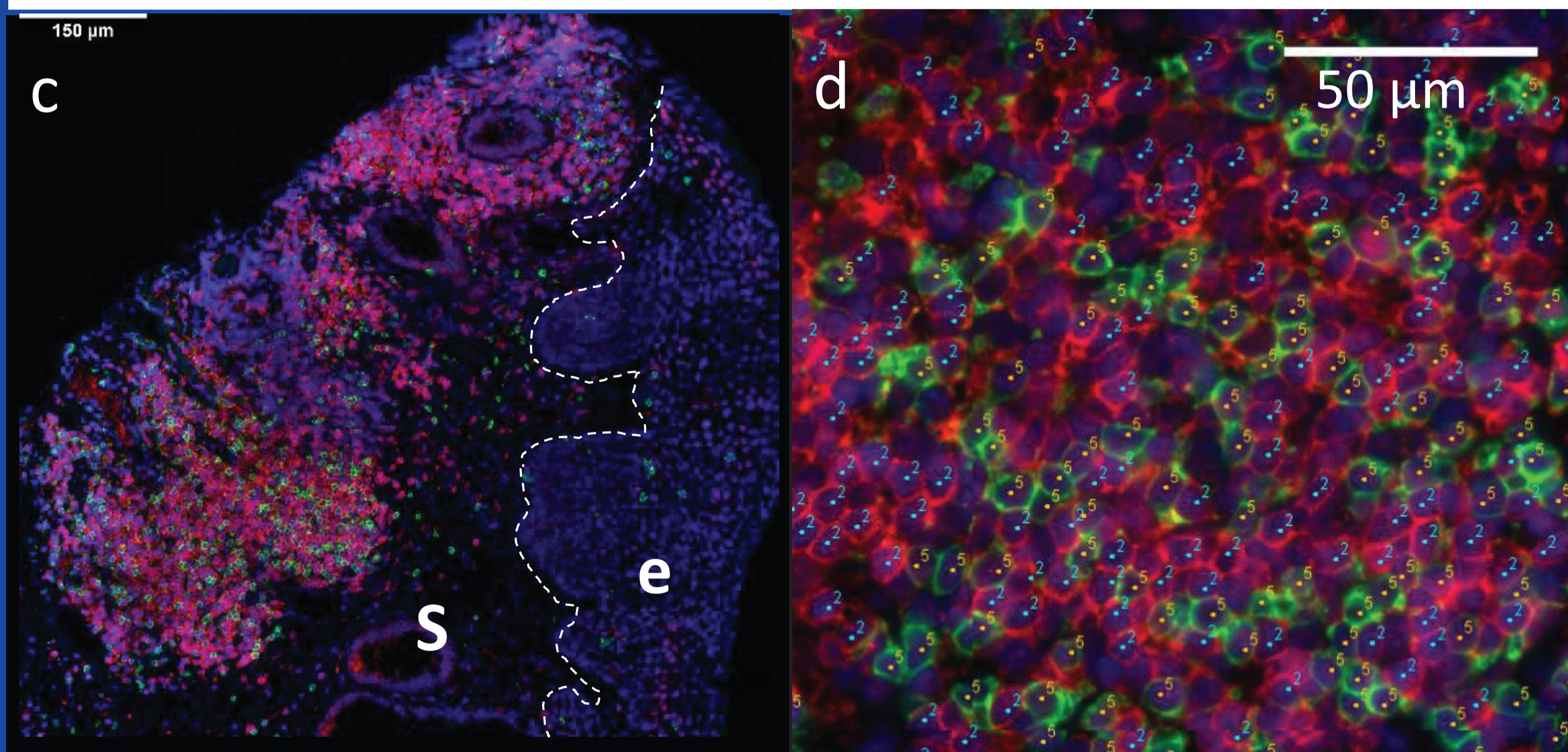
Introduction

T-cell lymphocytic aggregates in anal high-grade squamous intraepithelial lesions (HSIL) have not been previously described.



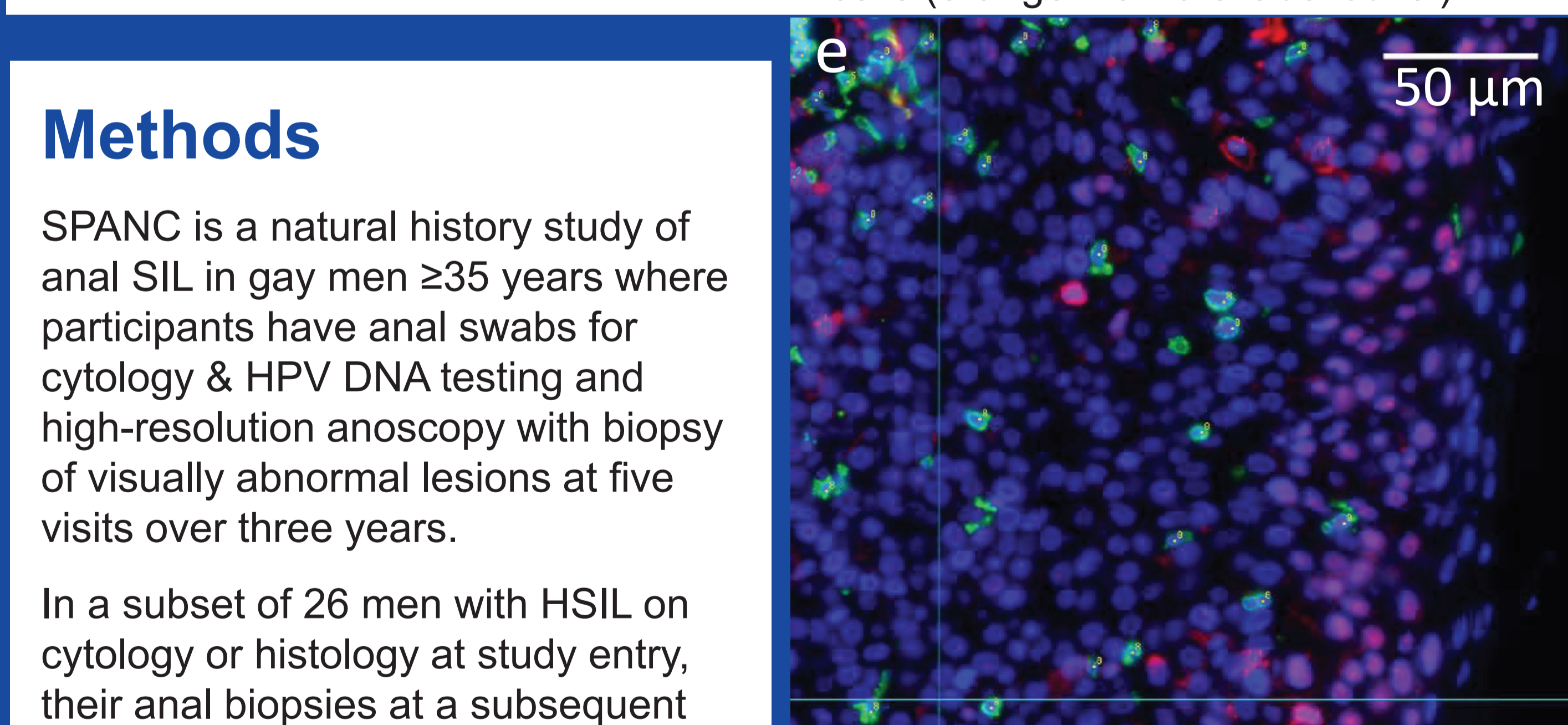
(a) H&E stained anal biopsy with lymphocyte aggregate.

(b) Same biopsy immunostained for CD4+ (red) and CD8+ (green) cells.



(c) Magnified box inset from panel (b) demonstrating stromal (s) and epithelial (e) compartments.

(d) Manual cell counting in the stromal compartment - CD4+ cells (cyan markers labelled '2') and CD8+ cells (orange markers labelled '5').

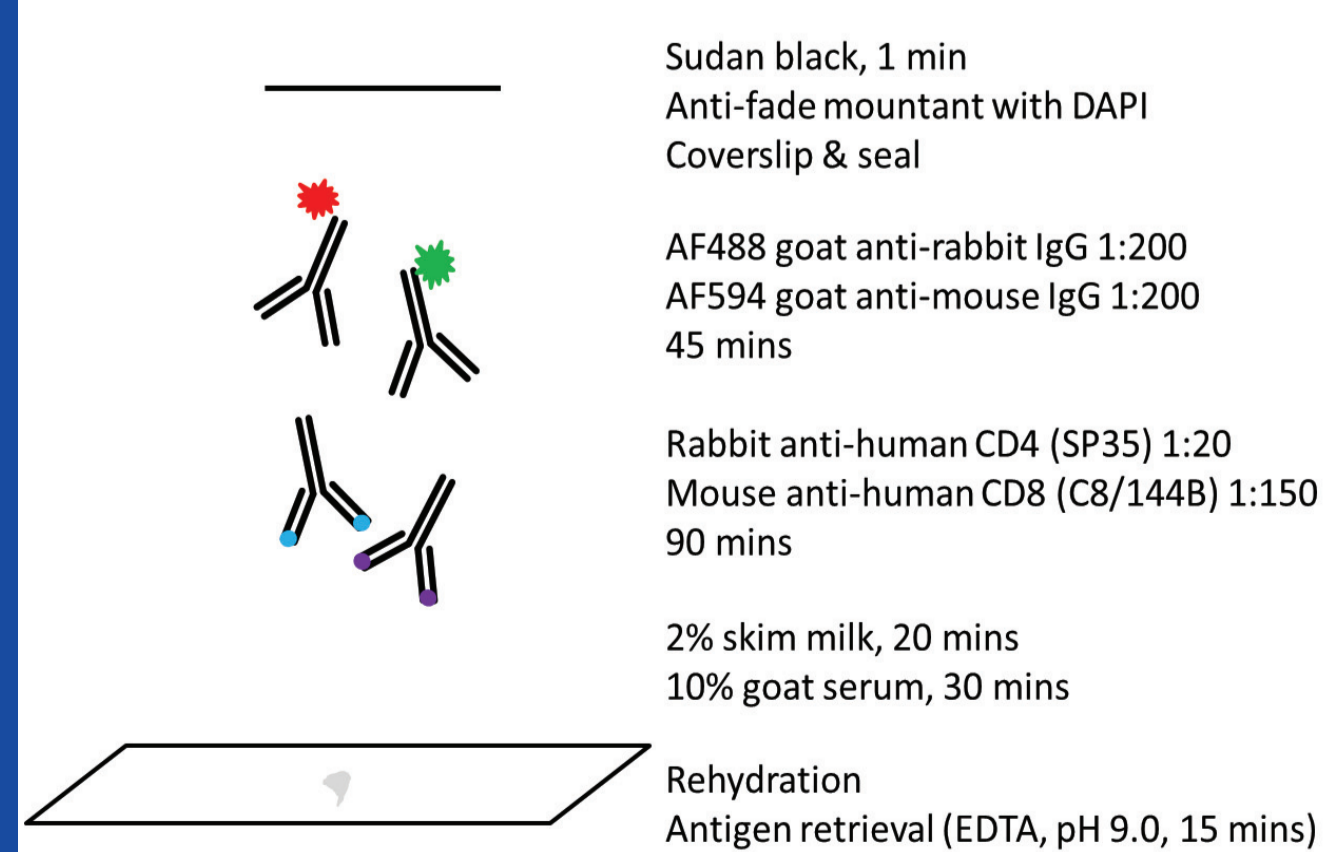


(e) Manual cell counting in the intraepithelial compartment - CD4+ cells (crimson markers labelled '4') and CD8+ cells (light yellow markers labelled '8').

Methods

SPANC is a natural history study of anal SIL in gay men ≥ 35 years where participants have anal swabs for cytology & HPV DNA testing and high-resolution anoscopy with biopsy of visually abnormal lesions at five visits over three years.

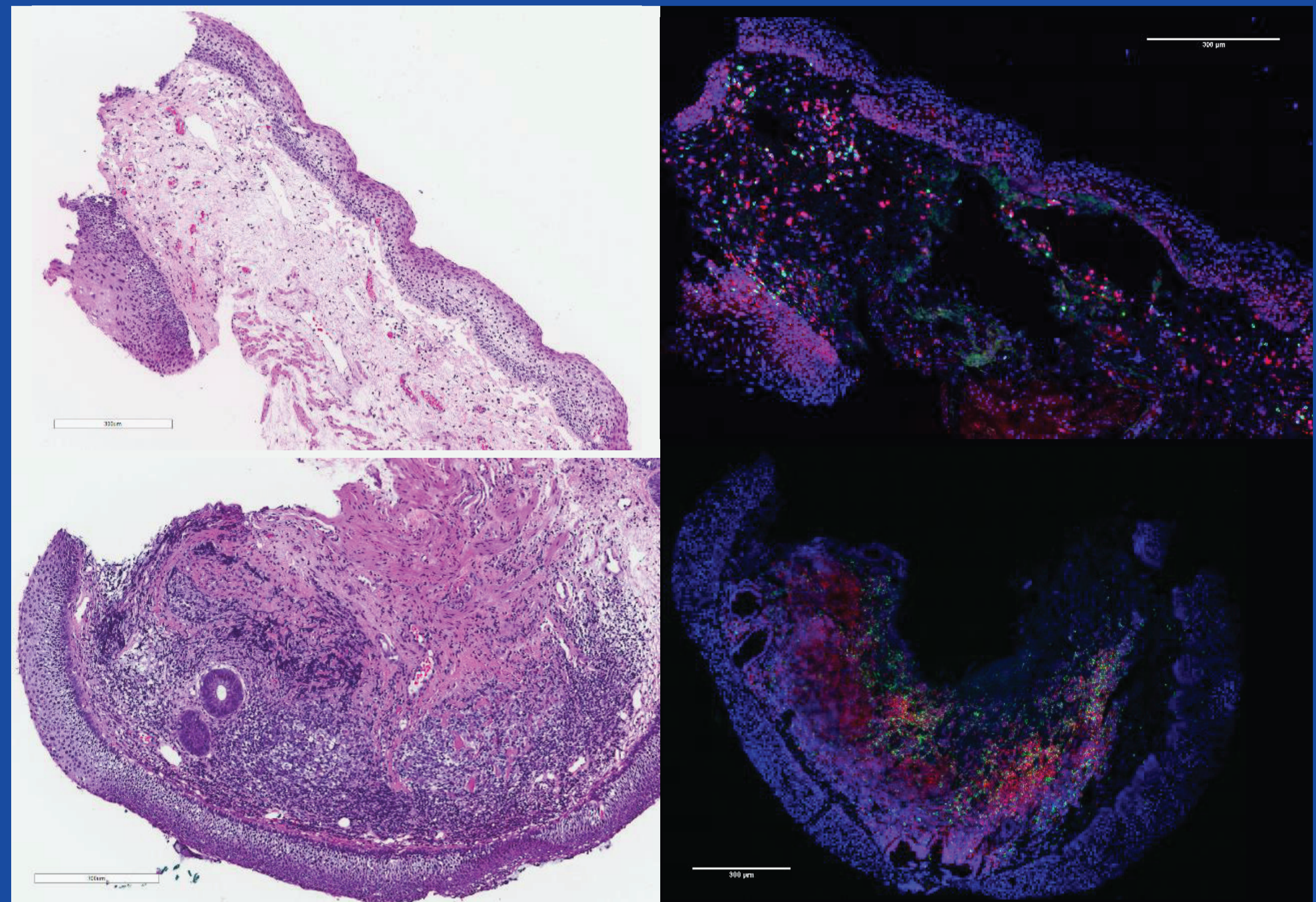
In a subset of 26 men with HSIL on cytology or histology at study entry, their anal biopsies at a subsequent visit ($n = 44$) were dual immunostained for CD4+ and CD8+ cells using the following protocol.



Whole slide imaging and CD4+ and CD8+ cell enumeration was performed as illustrated above. T-cell density was \log_{10} transformed. *t*-tests were used to compare means. A generalised linear model was used to determine factors associated with total T-cell density (biopsy-based with intra-subject adjustment).

Results

- 24 (55%) biopsies had lymphocytic aggregates on H&E
- Biopsies with lymphocytic aggregates had higher total T-cell densities compared to those without (mean 294 vs 141 cells/mm², $P=0.01$)



Representative biopsies without (top panel) and with (bottom panel) lymphocytic aggregates. H&E staining (left) with matching CD4/CD8 dual immunofluorescence staining on the right. All scale bars = 300 μm .

- Mean CD4+ T-cell density was 2.8 fold greater ($P<0.01$) in these aggregates but CD8+ T-cell density was only 1.7 fold greater ($P=0.08$)
- 26 (59%) biopsies had histological HSIL (AIN2 or 3)
- Higher total T-cell density was significantly associated with:
 - HSIL histology (OR 11.80, 95% CI 1.51 – 92.08, $P=0.02$) compared to biopsies without HSIL histology; and
 - Presence of anal HPV16 (OR 14.08, 95% CI 1.15 – 172.71, $P=0.04$) compared to absence of anal HPV16
- There was no association between presence of anal low-risk HPV types and total T-cell density

Conclusion

CD4+ T-cell enriched lymphoid aggregates are common in the anal mucosa of men with HSIL, and are associated with histological HSIL diagnosis and presence of anal HPV16. These immune cells may play a role in the natural history of anal HSIL and cancer.

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