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Automated Ki-67 LI assessment in prostate cancer using artificial intelligence in multiplex fluorescence immunohistochemistry

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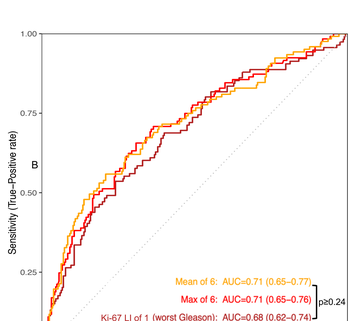
Introduction and Objectives

The Ki-67 labeling index (Ki-67 LI) is a strong prognostic marker in prostate cancer. Its analysis requires cumbersome manual quantification of Ki-67 immunostaining in at least 200 tumor cells.

Materials & Methods

To enable automated Ki-67 LI assessment in routine clinical practice, we have developed and validated a framework for automated Ki-67 LI quantification, which comprises three different artificial intelligence analysis steps and an algorithm for cell-distance analysis of multiplex fluorescence immunohistochemistry staining. The prognostic impact of the Ki-67 LI was tested on a tissue microarray (TMA) containing one sample each of 12475 prostate cancers. A “heterogeneity TMA” containing 3 to 6 samples from different tumor areas was used to model Ki-67 analysis of multiple different biopsies. Additionally, 30 biopsies were analyzed for method validation in brightfield and fluorescence staining.

Heterogeneity TMA analysis

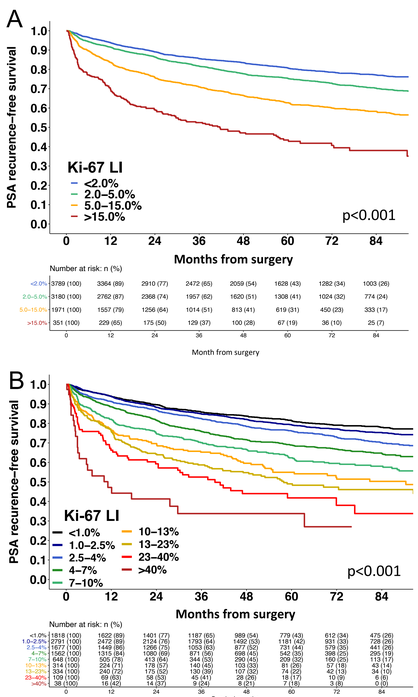


The analysis of the heterogeneity TMA revealed that the Ki-67 LI of the sample with the highest Gleason score (AUC:0.68) was similarly prognostic as the mean Ki-67 LI of all six foci (AUC:0.71 [p=0.24]).

RESULTS

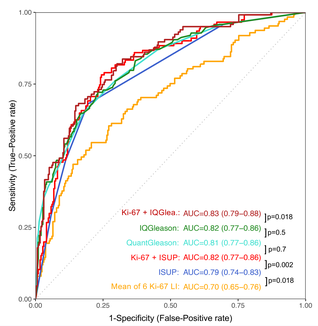
Classical prognosis TMA analysis

	Patient number (%)	Ki-67 LI (SD)	p-value
Total	11845 (100.0)	4.6 (6.5) Median 2.7	
PSA level			<0.001
<4 ng/ml	1475 (12.5)	4.2 (6.0)	
4-10 ng/ml	6963 (58.8)	4.4 (6.1)	
10-20 ng/ml	2487 (21.0)	5.1 (7.2)	
>20 ng/ml	849 (7.2)	5.3 (7.6)	
Missing data	71 (0.6)		
Pathological tumor stage			<0.001
pT2	7582 (64.0)	3.8 (5.3)	
pT3a	2646 (22.3)	5.2 (6.1)	
pT3b-4	1570 (13.3)	7.2 (10.1)	
Missing data	47 (0.4)		
Pathological nodal stage			<0.001
pN-	7299 (61.6)	4.8 (6.5)	
pN+	897 (7.6)	7.6 (10.7)	
Missing data	3649 (30.8)		
Gleason grade of biopsy specimen			<0.001
≤3+3	3959 (33.4)	3.4 (4.1)	
3+4	2383 (20.1)	4.1 (4.7)	
4+3	1058 (8.9)	5.2 (6.7)	
≥4+4	959 (8.1)	6.2 (8.3)	
Missing data	3486 (29.4)		
Gleason grade of prostatectomy specimen			<0.001
≤3+3	2174 (18.4)	3.0 (3.6)	
3+4	6219 (52.5)	4.1 (5.2)	
3+4 Tert.5	605 (5.1)	4.7 (5.2)	
4+3	1124 (9.5)	5.9 (8.0)	
4+3 Tert.5	854 (7.1)	6.8 (7.7)	
≥4+4	724 (6.1)	9.2 (13.2)	
Missing data	145 (1.2)		



The Ki-67 LI provided strong and independent prognostic information in 11845 successfully analyzed prostate cancers (p<0.001 each). The risk for PSA recurrence increased continuously along with an increasing Ki-67 LI, irrespective of how subgroups were formed: Four groups (A) and 9 groups (B) (p<0.001).

Performance of the Ki-67 LI compared to Gleason grades



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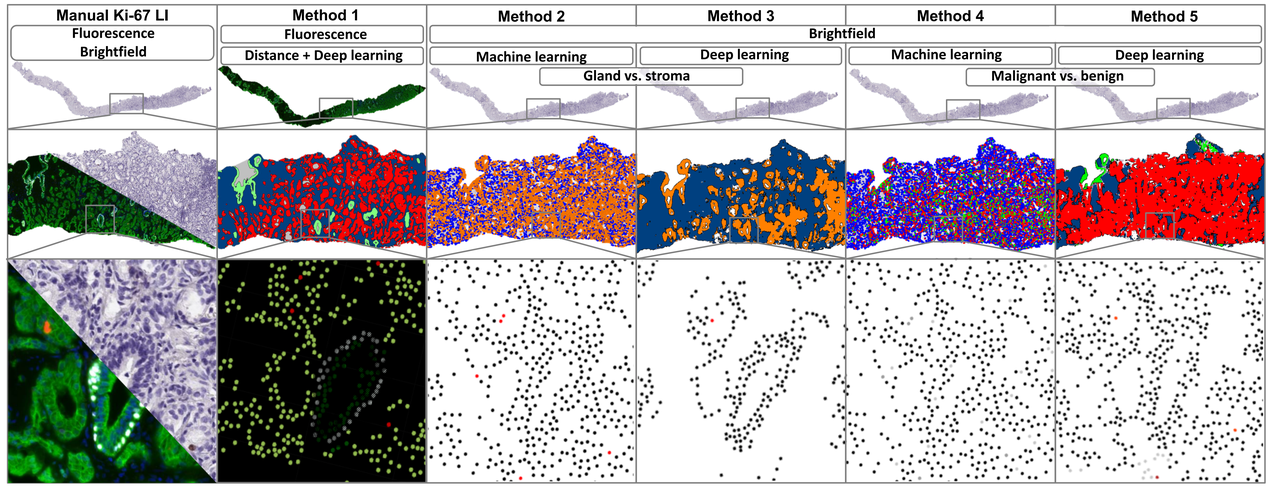
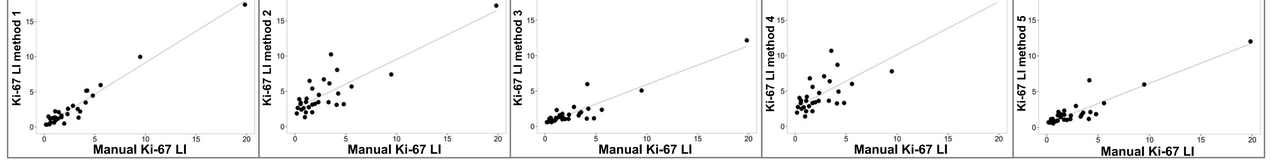
Automated Ki-67 LI quantification in prostate cancer biopsies





A comparison of the shown automated prostate cancer detection framework (Method 1) was compared to the manual Ki-67 LI and four different “classical” bright field based semi-automated Ki-67 analysis approaches (Method 2-5) on 30 prostate biopsies. To compare Method 1 with “classical” bright field IHC analysis a machine learning (ML) as well as deep learning (DL) framework was established. The fluorescence based automated Ki-67 LI quantification framework (intraclass correlation [ICC]: 0.97) showed a 11 % to 17 % superior concordance with the manual Ki-67 LI compared to the bright field-based methods (ICC: 0.80).

Conclusions

- The Ki-67 LI is a powerful prognostic parameter in prostate cancer that can be efficiently analyzed using **artificial intelligence** on **multiplex fluorescence IHC (mfIHC)**.
- In case of multiple cancer positive biopsies, the **sole analysis of the worst biopsy can be sufficient**.
- The successful assessment of the Ki-67 LI in more than 10’000 prostate cancers revealed an average **Ki-67 LI of 4.6%.**
- The comparison of classical bright field-based semi-automated Ki-67 quantification with mfIHC-based framework in prostate cancer biopsies revealed an up to **17 % higher concordance** with the manual Ki-67 LI.

Ki-67 PanCk

Conflicts of interest: The Ki-67 (MSVA 267M) and PanCK (MSVA-000R) antibody clones were provided by MS Validated Antibodies GmbH (owned by a family member of GS)

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