Digital-image analysis of tumor infiltrating lymphocytes in prostate cancer using automated whole slide imaging and tissue microarray

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Background

• There have been numerous reports showing that inflammation influences prostate cancer (PCa) development and that immune cells are among the primary drivers of this effect, leading to clinical trials testing immunotherapy drugs in PCa patients.

•Very few approved algorithms exist for quantifying tumor infiltrating lymphocytes (TIL) in prostate cancers, and the optimal methodology are currently unknown.

•Tissue microarray (TMA) is a powerful tool for high-throughput molecular analysis of tissues that is helping identify new diagnostic and prognostic markers in human cancers.

•One of the most common criticisms of tissue microarray is that the small cores sampled may not be representative of the whole tumor, particularly in heterogenous cancers such as prostate adenocarcinoma.

Objectives

• In this study, we propose to test the hypothesis that intratumoral heterogeneity is a major impediment to the use of TMA in the assessment of TIL in prostate cancers.

•Using whole slide imaging by virtual microscopy, we present the first spatial quantitative study of tumor infiltrating lymphocytes in 80 primary prostate adenocarcinomas and the corresponding TMA.

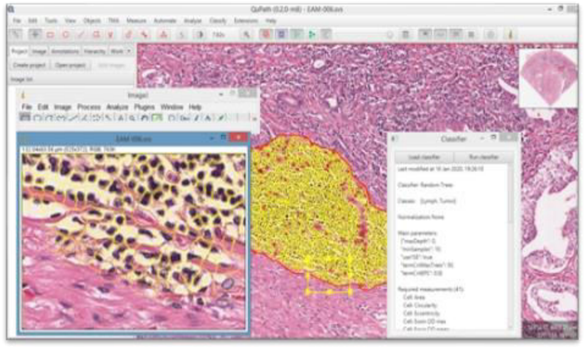
•Tissue Microarray based quantitative immune cell counts were validated by paired TIL quantification in whole-slide cohorts.

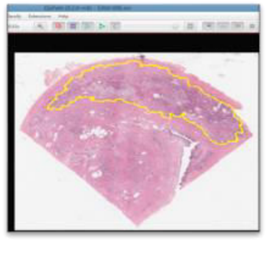
Design & Methods

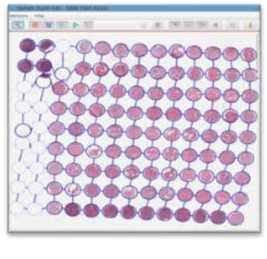
Eighty patients who were operated on for PCa in our institution between 2008 and 2018 were included in the study. All diagnoses were established according to classical histopathological criteria. Demographic parameters and the main characteristics of patients' tumors are reported in Table below:

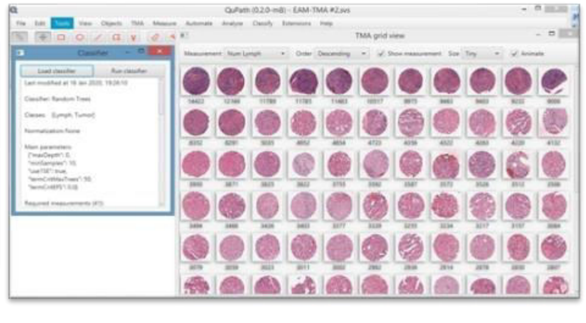
Category	Characteristic	n (%)
Race	Central American	80 (100)
Pre-treatment PSA (ng/mL)	<10	66 (82.5)
	10-20	12 (15)
	>20	2 (2.5)
Clinical Stage	T1c	51 (63.8)
	T2	3 (3.8)
	T2a	13 (16.3)
	T2b	1 (1.3)
	T2c	6 (7.5)
	T3a	1 (1.3)
	T3b	1 (1.3)
	Unknown	4 (5)
Biopsy Gleason Score	Grade 1 (3+3)	44 (51.3)
	Grade 2 (3+4)	19 (23.8)
	Grade 3 (4+3)	7 (8.8)
	Grade 4 (4+4)	9 (11.3)
	Grade 5 (4+5)	1 (1.3)
Pathological Stage	T2	13 (16.3)
	T2a	23 (28.8)
	T2b	7 (8.8)
	T2c	2 (2.5)
	T3a	26 (32.5)
	T3b	8 (10)
	T3x	1 (1.3)
Treatment fail	No	45 (56.3)
	Yes	35 (43.8)

- *The TMA was constructed by using a 1mm corer. Each tumor was represented in triplicate (Beecher Tissue Arrayer MTA-1, California, USA)*
- *Most representative H&E slide with corresponding TMA slides (80 prostate cancer cases) were scanned at 40X using XY scanner.*
- *The total number of aggregates per section, total area of Intra/peri-lesional size, and number of lymphocytes in each aggregate were determined using QuPath-0.2.0-m8 computer image analysis system.*



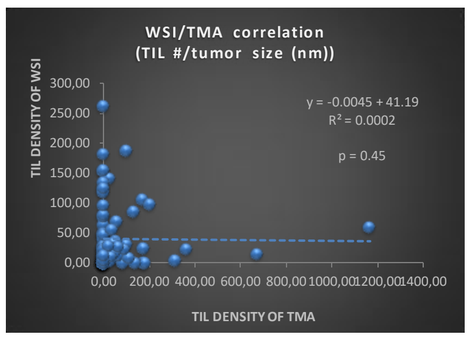


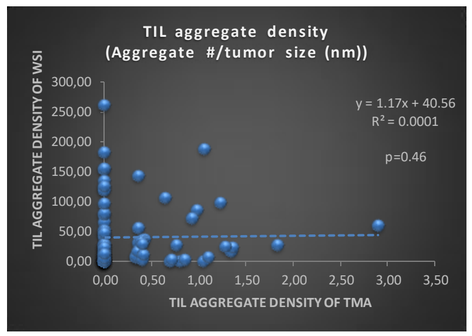




Results

We present the first quantitative study of tumor infiltrating lymphocytes in 80 primary prostate adenocarcinomas. The TMA method was validated against whole slides analysis. Spearman's rank correlation analysis was used to find possible correlation between WSI and TMA. In most patients both peritumoral and intratumoral TLS can be seen on wholes slides, but in TMAs the peritumoral TLS could not be evaluated at all due to the small size. No correlation was found between density of lymphocytes calculated from WSI and TMA respectively (r=-0.014). No significant differences were found between TMA and WSI in all subgroups (p values are between 0.09 and 0.98). Our results suggest that whole slide imaging and evaluation by virtual microscopy is irreplaceable for TIL quantification in PCa.

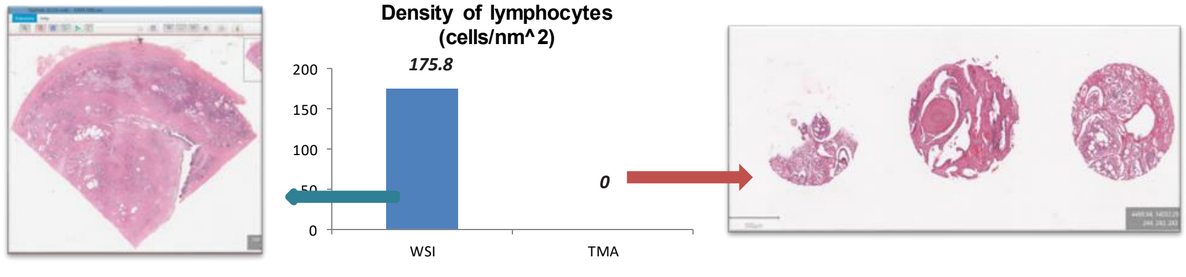




Density of lymphocytes (cells/nm^2) (Mean density ± Standard Deviation)	TMA	WSI*	WSI**	*P*-value*	*P*-value**
Pre-treatment PSA (ng/mL)					
<10 (n=66)	59.9±157.5	41±54.8	46.3±61.6	0.36	0.52
10-20 (n=12)	66.9±193.7	40.3±50.6	46.4±54.3	0.66	0.74
>20 (n=2)	11.1±19.2	88.7±87.9	90.5±89.7	-	-
Clinical Stage					
T1c (n=51)	50.2±118	35±77.6	40±49.9	0.39	0.57
T2 (n=3)	0.0±0.0	121.4±130.3	141.6±163.1	0.25	0.27
T2a (n=13)	48.4±76.5	43.7±57.2	49.3±62.4	0.88	0.98
T2b (n=1)	100.9±0.0	31.5±0.0	35.3±0.0	-	-
T2c (n=6)	227.2±461.2	30.0±27.3	32.3±28.5	0.33	0.33
T3a (n=1)	33.3±0.0	6.7±0.0	6.7±0.0	-	-
T3b (n=1)	0.0±0.0	78.0±0.0	79.6±0.0	-	-
Unknown (n=4)	25.7±30.8	65.5±77.5	69.5±77.1	0.47	0.44
Biopsy Gleason Score					
Grade 1 (n=44)	84±205.6	39.4±54.8	47.1±64.5	0.17	0.26
Grade 2 (n=19)	22.8±54.9	40.2±45.2	41.6±46.1	0.35	0.32
Grade 3 (n=7)	64.9±115.3	35.0±62.5	43.7±63.6	0.70	0.72
Grade 4 (n=9)	22.9±34.1	53.5±64.8	59.3±67.4	0.13	0.09
Grade 5 (n=1)	0.0±0.0	0.0±0.0	0.0±0.0	-	-
Pathological Stage					
T2 (n=13)	142.2±187.5	50.8±75.9	55.7±90.1	0.16	0.19
T2a (n=23)	72.9±241.3	31.5±37.0	39.4±45.5	0.41	0.51
T2b (n=7)	43.9±75.5	48.6±54.1	50.2±52.6	0.89	0.85
T2c (n=2)	22.5±31.9	18.4±2.3	23.6±9.7	0.89	0.98
T3a (n=26)	25.9±70.7	44.5±55.6	49.0±60.5	0.32	0.23
T3b (n=8)	30.1±64.3	21.6±24.7	26.5±25.4	0.77	0.90
T3x (n=1)	0.0±0.0	181.5±0.0	185.1±0.0	-	-
Treatment fail					
No (n=45)	65.7±182.7	38.9±55.4	44.6±63.1	0.34	0.46
Yes (n=35)	52.4±128.0	43.6±51.5	48.3±56.0	0.72	0.87
Total (n=80)	**59.9±159.3**	**41±53.2**	**46.3±59.3**	**0.32**	**0.48**

WSI : Density of lymphocytes-1 (cells/nm^2): Intra-lesional lymph number/tumor size of WSI*
*WSI**:Density of lymphocytes-2 (cells/nm^2): total lymph number/tumor size of WSI*
P-value: Paired sample t-test between TMA and WSI**
*P-value**: Paired sample t-test between TMA and WSI***

Hematoxylin and eosin staining of primary prostate adenocarcinoma from a 60-year-old male. ***Left)*** This whole slide image were specifically chosen to represent a spectrum of density of lymphocytes. Density of lymphocytes calculated is 175.8 cells/nm^2. ***Right)*** Three tissue cylinders with a diameter of 1 mm were punched from three sites. Density of lymphocytes calculated is 0. ***Middle)*** Comparison of TMA With WSI Results in the calculation of Density of lymphocytes (175.8 cells/nm^2 for WSI vs. 0 for TMA)



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Discussion

We present the first spatial quantitative study of tumor infiltrating lymphocytes in 80 primary prostate adenocarcinomas. Tissue Microarray based quantitative immune cell counts were validated by automated paired TIL quantification in whole-slide cohorts. Our results suggest that whole slide imaging by virtual microscopy is irreplaceable for TIL quantification, as a potential biomarker predicting and monitoring PCa treatment response. TMA analysis does not provide information of TME and TIL at peritumoral compartment of prostate cancer. Even while using three cores from each sample to construct TMA blocks it is not sufficient to allow for coverage of biologic heterogeneity of the infiltrating lymphocytes in the intratumoral compartment of prostate adenocarcinomas. One potential solution might be the creation of image microarrays (IMA) that would allow for capturing all of a tumor's morphologic variation on a single slide.