ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

Efficiency and Quality Improvement In Pathology Diagnostic by Using Computational Pathology: Software-Based Analysis of Perineural Invasion in Colon Carcinoma

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Submitted: 2023, Oct 10; **Accepted:** 2023, Nov 01; **Published:** 2023, Nov 24

Citation: Demirdag, D., Khacheishvili, M., Baidoshvili, A. (2023). Efficiency and Quality Improvement In Pathology Diagnostic by Using Computational Pathology: Software-Based Analysis of Perineural Invasion in Colon Carcinoma. *J Gene Engg Bio Res, 5*(3), 206-219.

Abstract

Introduction: *Many pathology laboratories are transitioning from diagnostics with glass slides to diagnostics with whole-slide images (WSI). One of the advantages of digital pathology is the possibility of using the software. Annotations made by software could increase the efficiency and the quality of pathologists´ work. In this research, annotations for nerves were used in WSI´s of colon carcinoma cases to help the detection of perineural invasion to improve the efficiency (time needed to find perineural invasion) and quality (the possibility of finding more perineural invasion in colon carcinoma cases and standardization) of the diagnostics.*

Materials & Methods: *4 pathologists got 148 colon carcinoma cases without annotations made by software to screen the slides for perineural invasion. After a minimum of 4 weeks of washing out, they got the same instances with annotations for nerves made by software to find perineural invasion. We compared the time they needed for the cases with and without annotations and the percentage of perineural invasion found with and without annotations.*

Results: *All 4 pathologists took advantage of the annotations made by software regarding the time they needed to conclude the perineural invasion, and the difference between the average time of all 4 pathologists without and with the use of software was statistically significant. All 4 pathologists found individual more perineural invasion. However, the average % of found perineural invasion without and with the help of software needed to be more substantial.*

Keywords: Digital Pathology, Software-Based Analysis, Colon Cancer, Quality Improvement, Efficiency Improvement.

Abbreviations

Labpon – Laboratorium Pathologie Oost-Nederland
WSI – Whole Slide Image
PIN – Perineural Invasion

1. Introduction

Digital pathology has become popular in the last decade and allows new possibilities in pathology diagnostics. The interest in this research field is growing rapidly. There are a couple of studies about the new feasibilities of digitalization with advantages and disadvantages [1-11]. However, the transition to digital pathology can make the clinical workflow easier and more efficient, and digitalization can bring new opportunities into digital diagnostics; the primary diagnostics still needs to be improved [12]. Different validation studies demonstrate the safety of digital diagnostics also. They present a high concordance between digital diagnosis and diagnosis with light microscopy, and Goacher at el. reported in their review an increasing concordance in the recent studies [13, 14] (Table 1).

92%	Digital slide and virtual microscopy-based routine and telepathology evaluation of routine gastrointestinal biopsy specimen	2003	Molnar et al., Semmelweis University, Budapest, Hungary (11)
95%	WSI for primary diagnosis in the gastrointestinal tract	2009	Al Janabi et al, Utrecht, NL (7)
95%	Concordance between digital pathology and light microscopy in general surgical practice: a pilot study of 100 cases	2014	Houghton et al., Belfast, Ireland (12)
92,4 %	The diagnostic concordance of WSI and light microscopy, review, and the result is weighted by the number of studies	2017	Goacher et al., University of Leeds, England (5)
97,8%	Validation of a WSI teleconsultation network	2018	Baidoshvili et al., Labpon Hengelo, Isala Zwolle, UMCG Groningen (3)

Table 1: Results of concordance studies from different years

With the attendance of deep learning software in pathology diagnostics, there are new possibilities to increase the efficiency and accuracy of the diagnosis [15]. In the time of personalized medicine, pathologists need to look after more different markers in a slide, and quantification is getting more important. Using software could make pathologists´ work more efficient and help standardize pathology diagnostics (decrease subjectivity).

Many companies are trying to develop software presenting annotations for pathologists to make diagnostics easier, more efficient, and more accurate [16,17]. The annotations are an area of interest (tumor cells, biomarkers, nerves, etc.). We assume that using the software could increase the efficiency and quality of pathologists´ work and reduce the chance of missing important prognostic features in a tumor, which could influence the treatment of patients and increase objectivity.

A perineural invasion is a form of metastatic spread of a tumor. It is an important factor in malignancies of the head and neck, pancreas, biliary tract, stomach, prostate, colon, and rectum [18]. The role of the perineural status, particularly in colon carcinomas, was debated.

According to recent protocols, perineural invasion is not required in the pathology report of colon carcinomas. Still, until 2013 it had to be mentioned in the information at Labpon Laboratory for Pathology in Hengelo, The Netherlands [19]. 38 perineural invasion cases were reported in 264 colon carcinoma cases in 2013 (14%).

TNM (tumor/node/ metastasis) staging is the standard for the pathology report and determines patients' treatment. One problem with this staging is that there could be differences in the outcome of patients with the same TNM stage. This suggests the importance of other prognostic factors, like perineural invasion and other characteristics associated with worse prognoses [20, 21] (Table 2).

Percentage	Authors/year/type of study	Comment
19%	Liebig et al/2009/retrospectieve studie (18)	colon (30%rectum, 25% ascending colon, 22% descending colon)
33%	Liebig et al./2009/review of the literature (22)	colorectum
16,7%	Huh et al./2010/retrospectieve studie (27)	colorectum
14%	**Labpon/2013**	**Colon/found by pathologists at Labpon**
18,2%	Knijn et al./2016/systematic review (21)	colorectum
26%	van Wyk et al. /2017/ review (28)	colon

Table 2: Average % PIN found in the colon in different studies

Liebig et al. described the perineural invasion as significant as the lymphovascular invasion of some malignancies. They reported up to 33% perineural invasion in colorectal carcinoma at the time of resection. They reported an average of 19% perineural invasion in the colon, tumors in the ascending colon (25%), and tumors of the descending (22%) colon had more perineural invasion rates than transverse colon and sigmoid. They mentioned perineural invasion as an independent predictor of outcome in colorectal cancer [22].

Knijn et al. reported in their meta-analysis of 58 articles that perineural invasion is a strong prognostic factor in colorectal cancer and indicates a poor prognosis. In this analysis, the incidence of perineural invasion was 18,2% in colorectum.

Poeschl et al. found a correlation between aggressive phenotype and perineural invasion and designated PIN as an independent prognostic factor in colon cancer. They referred to disease progres-

sion in more than 90% of patients with a perineural invasion-positive status compared to 32% of patients with a perineural invasion-negative status [23].

These and similar studies still suggest the importance of the perineural status of malignancies in the colon [24-25]. Perineural invasion is difficult to recognize in the colon; furthermore, it is an underestimated feature, and due to underreporting, understanding the significance remains a problem [26].

Due to the difficulty of finding perineural invasion, it is time-consuming for pathologists. Staining with S100 could be helpful in this process, but the attendance of software-based analysis could simplify this, even in HE-stained WSIs. Using annotations for nerves made by software seek the pathologist's attention to the important areas in the WSI, reducing the chance of missing perineural invasion and leading to a shorter screening time of the images. These two factors are important for improving the quality and efficiency of pathology diagnostics.

2. Materials and Methods

We choose perineural invasion in colon carcinoma cases for this study. We used annotations of nerves made by software to help detect perineural invasion. In this project, we wanted to prove that software annotations improve the diagnostics' efficiency and quality. As a parameter for efficiency, we used the exact time the pathologist needed to conclude perineural invasion (yes/no).

148 cases of colon carcinoma were chosen for the research. The cases were selected blindly from the routine pathology practice at Labpon. Each case contained 2 deidentified whole slide images stained with HE. From the 148 cases, 3 included 1 WSI, and the others contained 2 WSI. The preselection of the WSI´s per case was done blind. We did not have any inclusion or exclusion criteria. The slides stained with HE were scanned at Labpon with an IntelliSite ultrafast scanner at 40x.

A simple protocol with instructions to the pathologists was used to provide the same environment.

The pathologists were working on the same computer provided by Philips®. A hardware type HP Z240 and a monitor type Dell Ultrasharp 27 (U2717DWh) were used. For navigation and zooming, a general mouse from HP® was used, and 2 of the pathologists used an associate pen-tablet for navigation (Wacom Intuos PRO S®). This was used only for navigation in the WSI; they used the mouse for zooming. (Computer specifications: Table 3.1; 3.2; 3.3).

Manufacturer	**HP Inc.**
Model	HP Z240 SFF Workstation
Processor	Inter(R) Core (TM) i7-6700 CPU @ 3.40GHz 3.40 GHz
Installed memory	16,0 GB
Type of system	64 bits

Table 3.1: System

Windows 7 Professional
Copyright © 2009 Microsoft Corporation. All rights reserved.
Service Pack 1
Product-id:00371- OEM-8992671-00008

Table 3.2: Windows

Type	DELL U2717D
Resolution	2560 x 1440

Table 3.3: Monitor

The cases were stored on 2 different 4-terabyte discs provided by Philips®, one for the set without annotations made by software and one for the set with annotations made by software. The disc was connected to the SS output of the computers.

The WSIs were opened and viewed in Qupath®. The software Qupath® had to be installed on the computers. Qupath® is a whole slide image viewer and an image analysis software. Different tools are integrated into this software for visualization, annotation, batch processing, and image analysis.

Labpon Laboratory and Philips® are working on developing software for digital pathology. In our research, one of the pathologists made a training set of slides with annotations (highlighting) for nerves. Each training case contained 1 WSI stained with HE and another WSI stained with S100 (in the preparation, these were cut directly behind each other). The software was trained with these slides. The algorithm for the software for detecting nerves was

made at Philips® with 90% sensitivity and 70% specificity.

In our project, we were working with 4 pathologists. All of them had the same experience with digital diagnostics.

At first, 148 colon carcinoma cases were presented to each pathologist without annotation (Figure 1,2).

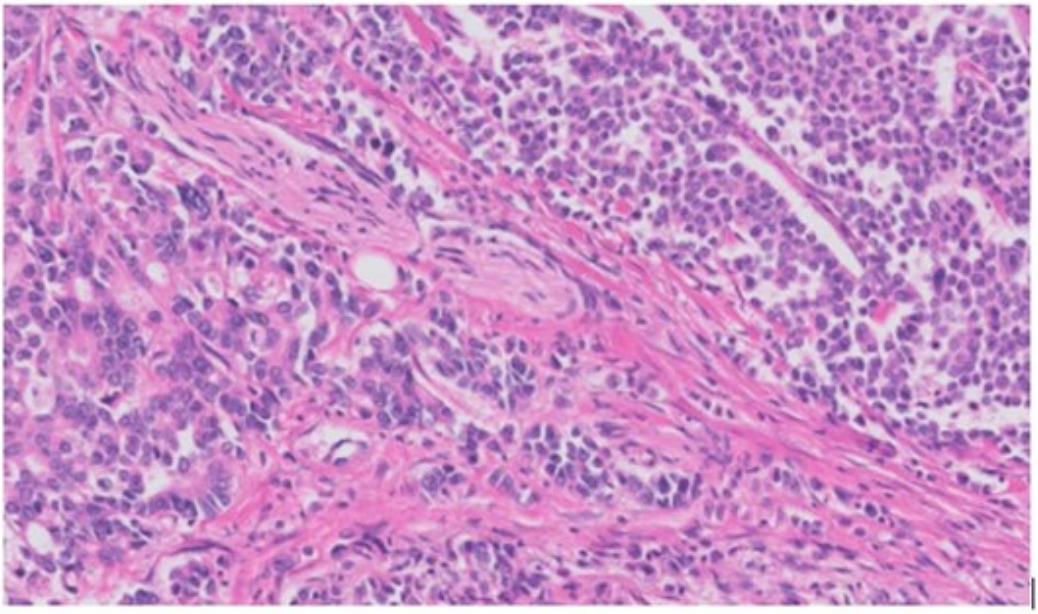

Figure 1. Part of a WSI without annotation of the nerve (high power)

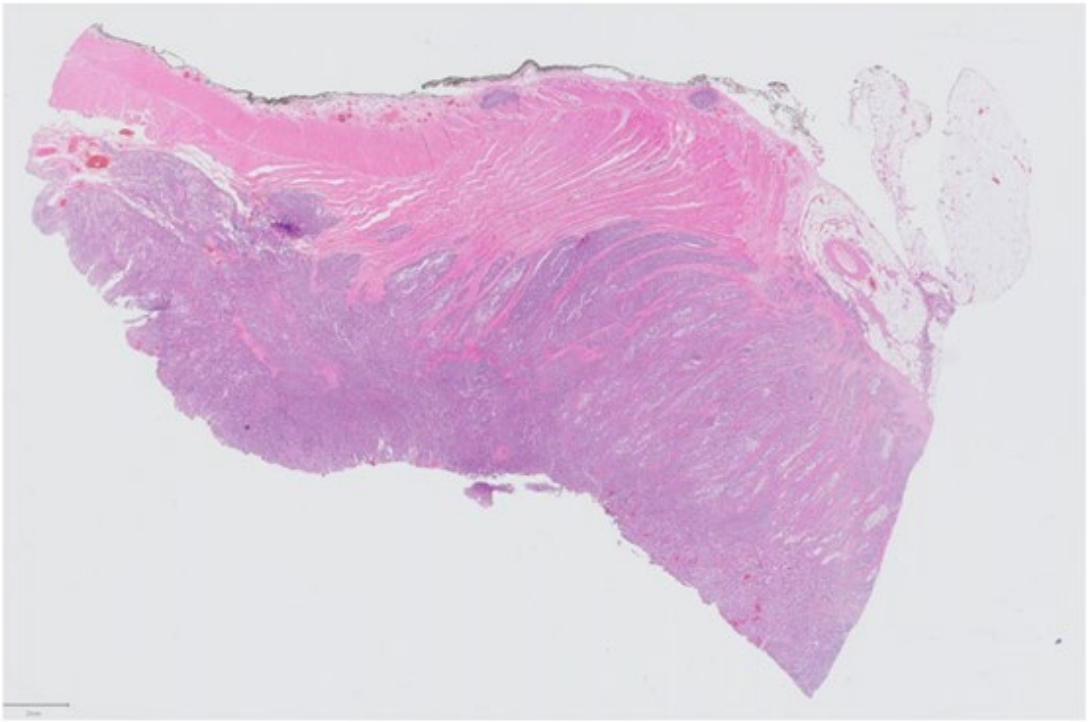

Figure 2. WSI without annotation (low power)

They had to screen the cases (contained a maximum of 2 WSI´s) for perineural invasion, and the time needed for the conclusion (presence or absence of perineural invasion) was measured. For measuring the time, a simple stopwatch was used. We were looking for 2 parameters: perineural invasion YES or NO, and time in minutes (2 decimals). If they found one perineural invasion, they could stop with screening and proceed with the next case. They wrote their results immediately in the chart we had prepared before. The pathologists had to come 4-5 times for a couple of hours to finish the list of 148 cases.

After a minimum of 4 weeks of washing out period, the same 4 pathologists got the same 148 cases with annotations of the nerves made by software (Figure 3,4). The sequence of the cases was changed, and a mix was made in blocks of 25 cases. We used the same chart in the second round and looked for the same parameters. In the second round, the pathologists looked only at the annotated areas made by software (nerves), and they had to conclude about perineural invasion. They checked those annotated areas, which could be important for the diagnosis.

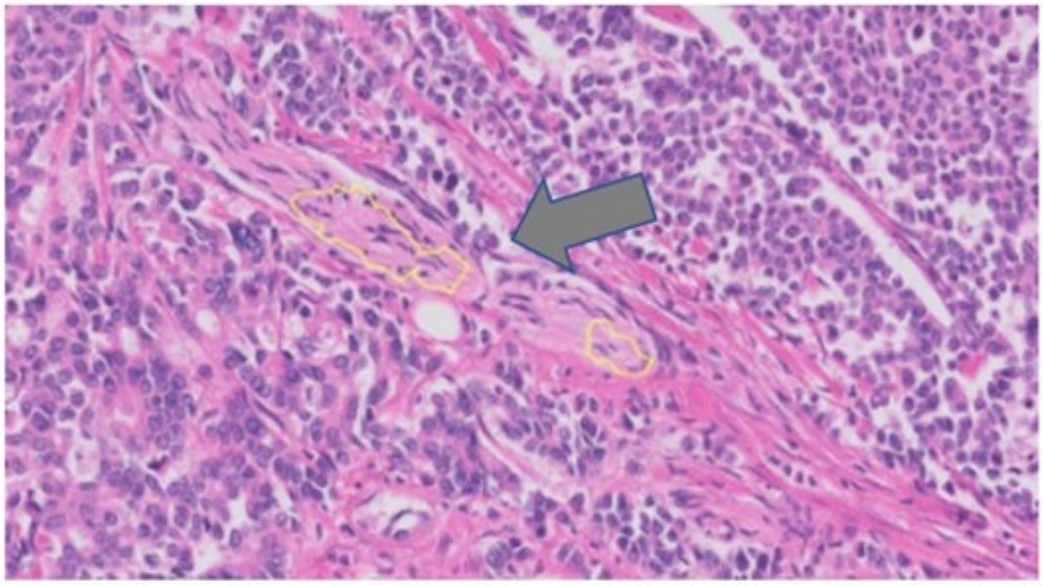

Figure 3. Part of a WSI with annotation of the nerve (high power)

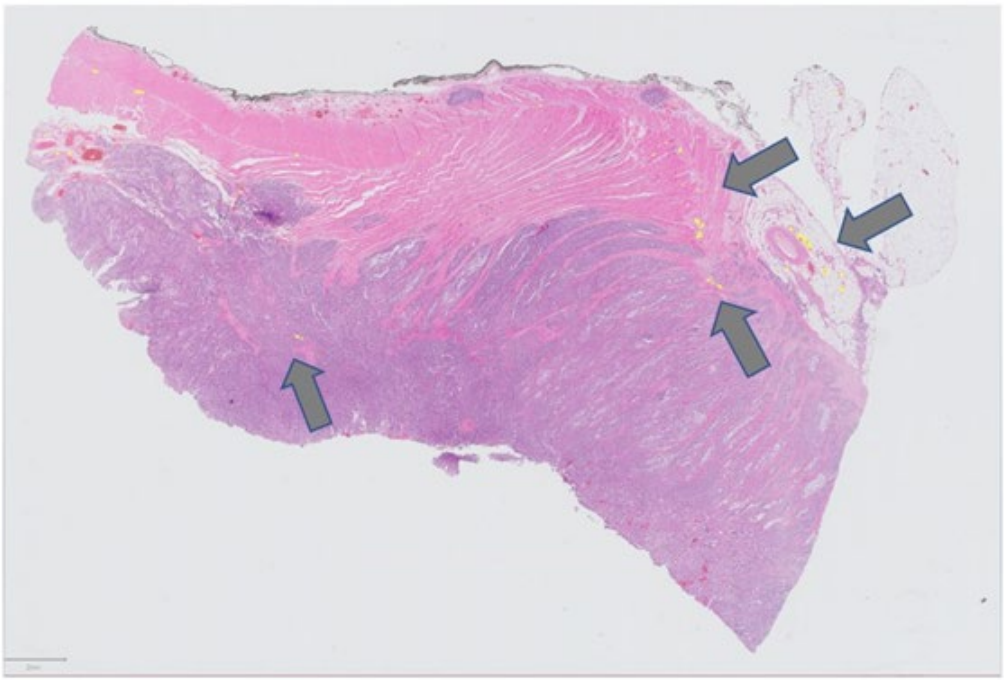

Figure 4 WSI with annotation (low power)

The results were analyzed in GraphPad (version 6.0). The average time per pathologist was calculated for round 1 (without annotations) and round 2 (with annotations made by software). This calculation was made in GraphPad. The percentage for perineural invasion was calculated per pathologist without and with annotations. The concordance between the diagnosis (slide containing perineural invasion or not) without and with annotations was analyzed individually, and the concordance between the 4 pathologists with and without annotations was also analyzed.

The result was discussed anonymously in a meeting of the digital pathology group.

3. Results

The average screening time per case for perineural invasion without annotations by the software was 2 minutes and 15 seconds (the average of all pathologists). With software, this average screening time per case could be reduced to 57 seconds (Table 4). With a Mixed Model analysis in SPSS (IBM SPSS Statistics version 25), the p-value was 1.07x10-116, < 0,001. This is statistically significant.

Pathologist	Average time needed without software	Average time needed with software	% perineural invasion found without software	% perineural invasion found with software
1	**1:54**	**0:53**	**12%**	**20%**
2	**1:49**	**1:02**	**14%**	**16%**
3	**3:48**	**1:03**	**8%**	**14%**
4	**1:35**	**0:50**	**26%**	**27%**
Average time/% perineural invasion of all pathologists	**2:15**	**0:57**	**15%**	**19%**

Table 4: Summary of results (comparing the time identifying PIN with and without software)

All 4 pathologists were taking advantage of their average screening times using software (chart 5.1.1; 5.2.1; 5.3.1; 5.4.1 and Table 4).

Results of Pathologist 1:

Time without software	Time with software
1:54	0:53

Table 5.1.1. Average time needed to conclude perineural invasion per case (min. sec.)

Results of Pathologist 2:

Time without software	Time with software
1:49	1:02

Table 5.2.1. Average time needed to conclude perineural invasion per case

Results of Pathologist 3:

Time without software	Time with software
3:48	1:03

Table 5.3.1. Average time needed to conclude perineural invasion per case

Results of Pathologist 4:

Time without software	Time with software
1:35	0:50

Table 5.4.1. Average time needed to conclude perineural invasion per case

The average percentage of found perineural invasion by all pathologists increased from 15% to 19%. With the Wilcoxon Sign Rank test (SPSS), a p-value of 0,125 was calculated, which is insignificant. However, all 4 pathologists found more perineural invasion using software (chart 5.1.2; 5.2.2; 5.3.2; 5.4.2 and Table 4).

Perineural invasion YES without software	Perineural invasion YES with software
18 cases	29 cases
12%	20%

Table 5.1.2. Found perineural invasion with and without software

Perineural invasion YES without software	Perineural invasion YES with software
21 cases	23 cases
14%	16%

Table 5.2.2 Found perineural invasion with and without software.

Perineural invasion YES without software	Perineural invasion YES with software
12 cases	20 cases
8%	14%

Table 5.3.2. Found perineural invasion with and without software

Perineural invasion YES without software	Perineural invasion YES with software
38 cases	40 cases
26%	27%

Table 5.4.2. Found perineural invasion with and without software

There is a high intra-and interobserver variation in reporting perineural invasion.

The concordance between the same pathologists reporting perineural invasion with and without software and between the 4 pathologists is diverse (charts 5.1.3; 4.2.3; 4.3.3; 4.4.3; and Table 6).

Found perineural invasion only without software	Concordances	Found perineural invasion only with software
7	11	18

Table 5.1.3. Number of found perineural invasions only without software/ number of found perineural invasions without and with software/ number of found perineural invasions only with software

Found perineural invasion only without software	Concordances	Found perineural invasion only with software
12	9	14

Table 5.2.3. Number of found perineural invasions only without software/ number of found perineural invasions without and with software/ number of found perineural invasions only with software

Found perineural invasion only without software	Concordances	Found perineural invasion only with software
5	7	13

Table 5.3.3. Number of found perineural invasions only without software/ number of found perineural invasions without and with software/ number of found perineural invasions only with software

Found perineural invasion only without software	Concordances	Found perineural invasion only with software
19	19	21

Table 5.4.3. Number of found perineural invasions only without software/ number of found perineural invasions without and with software/ number of found perineural invasions only with software

There was positivity for perineural invasion reported in 34% of the 148 colon carcinoma by at least 1 pathologist without software. 66% of the 148 cases were negative for perineural invasion, whereas none of the pathologists reported positivity for perineural invasion (Figure 5,6). 4% (6 cases) of the positive cases (based on 34%) had 100% concordance between the 4 pathologists. 3% (4 cases) of the positive cases were reported by 3 of the pathologists, 7% (10 cases) by 2 of the pathologists and 20% (29 cases) of the positive cases were reported only by one of the pathologists (Table 6).

Positive cases Without software	Case number	Number of cases	Percentage
Cases found + by 4 pathologists	33, 51, 58, 71, 84, 99	6	4%
Cases found + by 3 pathologists	37, 46, 68, 81	4	3%
Cases found + by 2 pathologists	3, 5, 31, 40,72, 86, 97, 102, 114, 136	10	7%
Cases found + by 1 pathologist	6, 11, 13, 15,16, 17, 18, 19, 24, 27, 34, 35, 36,38,61, 92, 94, 95, 98, 101, 102,103, 104, 108, 111, 124,132, 133, 141	29	20%

Table 6: Analysis with case numbers without software.

Analysis of results with and without software

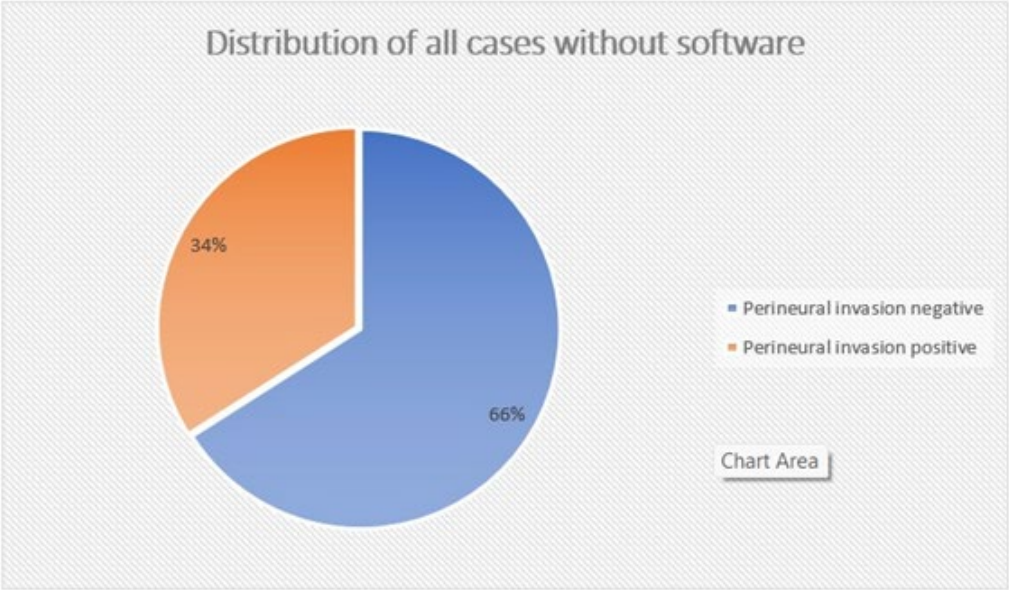

Figure 5. Analysis of cases without software.

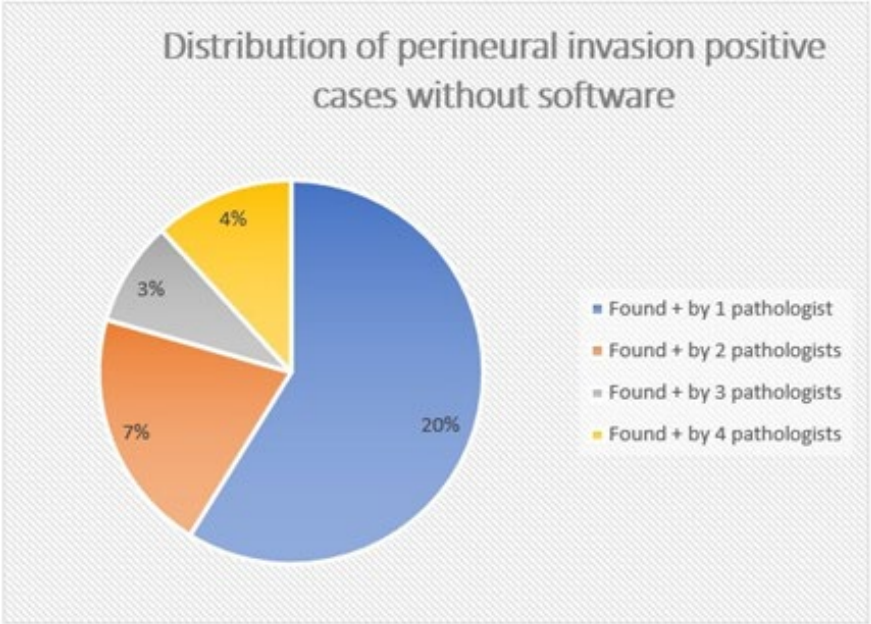

Figure 6. Concordance of the perineural invasion-positive cases without software (based on the 34%).

There was positivity for perineural invasion reported in 35% of the 148 colon carcinoma by at least 1 pathologist with software. 65% of the 148 cases were negative for perineural invasion (none of the pathologists reported positivity for perineural invasion). This is similar to the distribution of perineural invasion positive/negative cases without software, but there is a difference between the positive cases without and with software. 5% (7 cases) of the cases (based on 35%) had 100% concordance between the 4 pathologists. 6% (9 cases) of the positive cases were reported by 3 of the pathologists, 12% (17 cases) by 2 of the pathologists and 12% (17 cases) of the positive cases were reported only by one of the pathologists (Table 7).

Positive cases with software	Case number	Number of cases	Percentage
Cases found + by 4 pathologists	46, 71, 81, 98, 99, 141, 146	7	5%
Cases found + by 3 pathologists	31, 61, 68, 72, 84, 102, 103, 129, 136	9	6%
Cases found + by 2 pathologists	13, 15, 16, 29, 33, 37, 40, 52, 54, 92, 114, 116, 124, 126, 127, 137, 144	17	12%
Cases found + by 1 pathologist	2, 10, 14, 16, 30,35,36, 44, 50, 55, 58, 65, 86, 97, 112,124,139 140, 144,145	17	12%

Table 7: Analysis of case numbers with software.

There were 20 cases reported positive by at least 3 pathologists with or without software. From these 20 cases, 12 cases showed an improvement in the recognition by pathologists with software, 5 cases showed a decrease, and 3 cases were totally concordant with and without software. There are 2 cases where pathologists could not find any perineural invasion without software, and the same cases were reported positive by all pathologists with software (Table 8).

Case number	Several pathologists found perineural invasion without software	Several pathologists found perineural invasion with software	Description
31	2	3	
33	4	2	
37	3	2	*1
46	3	4	

51	4	1	
58	4	1	
61	1	3	*2
68	3	3	*3
71	4	4	
72	2	3	
81	3	4	
84	4	3	*4
98	1	4	
99	4	4	
102	2	3	
103	1	3	
129	0	3	
136	2	3	
141	1	4	
146	0	4	

Table 8: Comparison of the cases reported positive by 3 or more pathologists without and with software. (Increase in recognizing with software: ⬆; Decrease in recognizing with software: ⬇; software Concordance between with and without software: no sign
*1 One of the 3 pathologists missed it with software
*2 The pathologist who found it without software could not find it with the software
*3 The pathologist who has found it without software missed it with software, and one of the pathologists who have not found it without software could find it with software
*4 One of the 3 pathologists missed it with software

4. Discussion

The most important aim of this study was to demonstrate, that with the use of annotations made by software, pathologists can improve their efficiency in everyday diagnostics. Our results confirm our hypothesis that pathologists could work faster with software. It can be helpful if they are looking for features that are difficult to recognize, like perineural invasion in colon carcinoma. The average time that pathologists saved with software was 78 seconds per case, a decrease of 41%, from 2 minutes 15 seconds without software to 57 seconds with software. This could save for Labpon Laboratory for Pathology in Hengelo, The Netherlands, 6 hours and 12 minutes work in 2013 only for assessing perineural invasion in colon carcinomas.

Litjens et al. reported promising results in improving accuracy and efficiency with deep learning in diagnosing prostate and breast cancer in 2016. No exact numbers were mentioned regarding the time and what pathologists needed for the diagnosis.

An earlier study in 2014 confirmed the effectiveness of annotated slides in dermatology education and concluded that annotated whole-slide images have a future; however, this study focused on education and not on primary diagnosis.

We could not find another study reporting efficiency improvements in software-based pathology diagnostics.

Our study results show a high intra-and interobserver variation in the assessment of perineural invasion, according to the review of van Wijk et al. [27,28]. The difficulty of recognizing perineural invasion could be one of the reasons for this variation. Inflammatory environment, mucinous pools could cause an unclear image. Another problem is that there needs to be a standard definition of perineural invasion. More definitions are acceptable and created by different authors (Table 9).

Definition	Author/Year
Perineural invasion is a tumor cell invasion in, through, and around the nerve.	Batsakis et al./1984
Tumor cells inside de perineurium in the Auerbach plexus adjacent to the tumor front	Fujita et al./2007
Tumor cells close to neural structures involving at least 33% of the neural circumference or tumor cells within any of the 3 layers of the nerve sheath.	Liebig et al./2009
The cancer spread along the plexus of Auerbach (=intramural), tumor cells invading or spreading along nerve fascicles to the muscularis propria	Ueno et al./2013

Table 9: Different definitions of perineural invasion

Another possible reason for the variations in our study, next to the difficulties of recognizing, is that pathologists were asked to handle like in their average diagnostics. We did not assign an exact definition of perineural invasion.

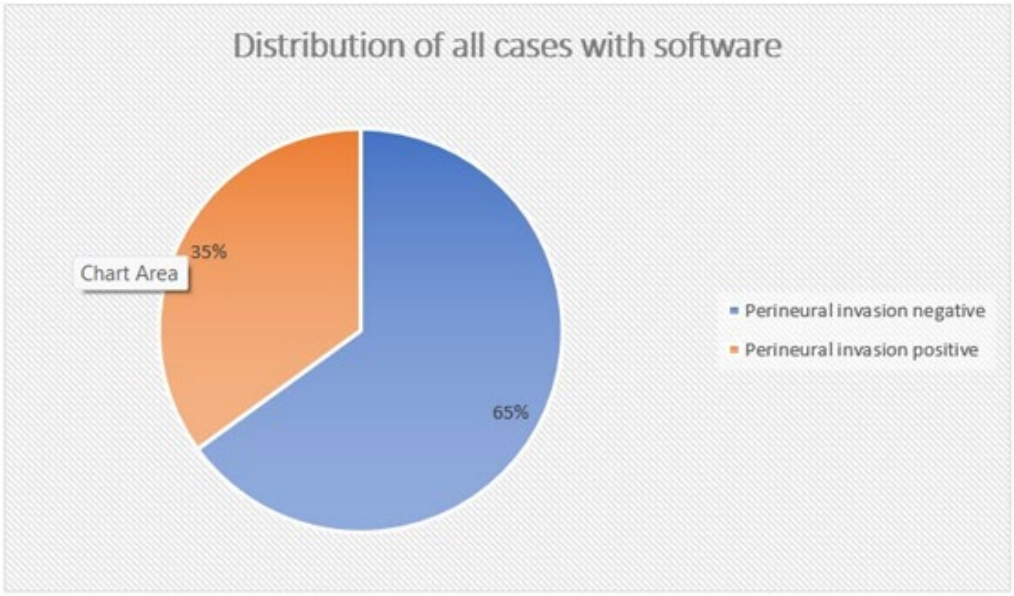

Figure 7. Analysis of all cases with software.

The pathologists in this study found an average of 15 % perineural invasion in the 148 colon carcinoma cases without using software (Table 4). This correlates with the 14 % that was found in the year 2013. Although the last is based on 264 cases of the year and diagnosed by different pathologists, the cases probably contained more than 2 WSIs.

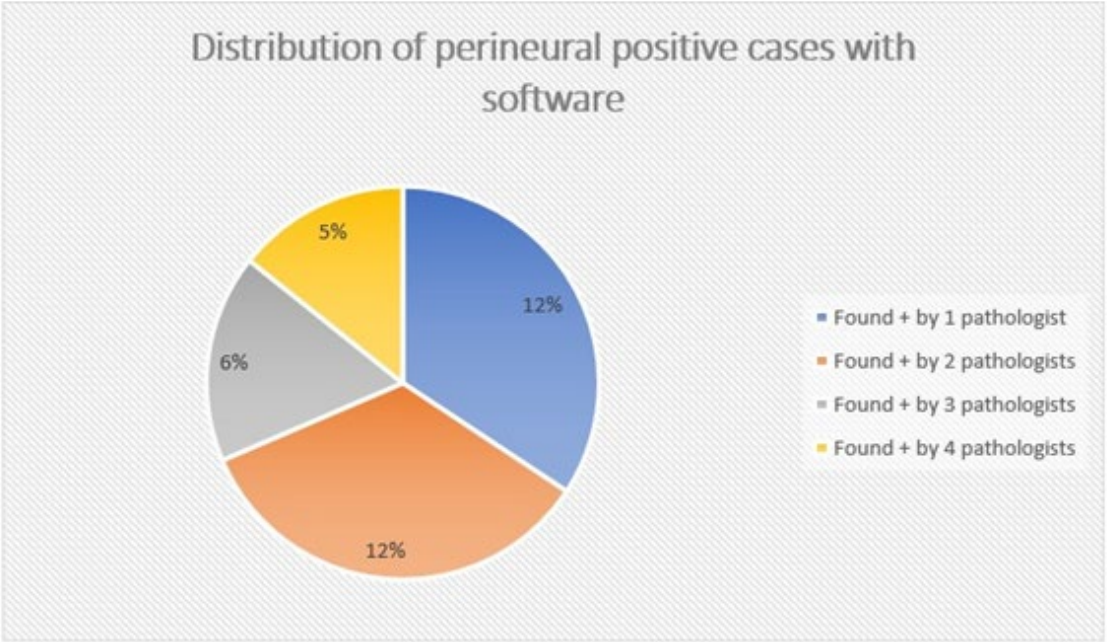

Figure 8. Concordance of positive cases with software (based on 35%).

4 % improvement was achieved with software; however, there was no statistically significant difference. The 19 % perineural invasion found with software falls in the range described in the literature, between 16,7 and 33%, and the % without software differs by only 1 %. The assessment in most of these studies was based on the colon and rectum. The incidence of perineural invasion in the colon is relatively low, and the perineural invasion rates in the colon are lower than in the rectum. This could establish the lower finding rates in this study.

Using annotation made by software could help pathologists recognize perineural invasion and other features in WSIs. As a result, in the 20 cases reported positive for perineural invasion by at least pathologists, pathologists could book 42% more improvement in finding perineural invasion (12 cases) than impairment (5 cases).

We can assume that using software in pathology diagnostics has a future, not only in the annotations made by software but in other deep learning options too: quantifying features and protein markers, measuring distances, localization of components, counting mitotic figures and nuclei, TMA analysis, analysis of tumor/ stroma ratio, qualification/grading, quality control of HE and IHC slides. The list of options is still growing.

The 4 pathologists found in the set of 148 annotated colon carcinoma cases, in some cases, a lot of false positive annotations, a few false negative annotations, some of the WSIs were not annotated, or there were not enough annotations. The sensitivity of the software was 90%, and the specificity was 70%. Changes in the sensitivity and specificity of the software could reduce false negative and false positive annotations.

Declarations

Ethics Approval and Consent to Participate

We want to inform the editorial team that this research was conducted in the laboratory of LabPON (Laboratorium Pathologie Oost-Nederland). The laboratory's scientific committee reviews issues related to the planned research (including bioethics). Research shows how different technologies (conventional or digital microscopes; software analysis) increase efficiency and quality in the diagnostic process. This process was monitored during a routine diagnostic process. Even during the routine diagnostic process, the patients who leave the material for the diagnosis to the laboratory sign a consent, implying that their tissues and biological material may be used during various studies. In addition, complete DE identification takes place in processing patient tissue and creating digital whole-slide images or non-digital slides, which also protects the principle of medical secrecy and confidentiality. However, the article does not directly address and discuss the characteristics of the patient's tissue and the diagnosis. Still, the time spent in the diagnostic process, identifying key features, and their comparison were essential for the study. Accordingly, the article does not provide diagnoses (already existing) but uses statistical data on time spent by the pathologist in reading and identifying key features during the diagnostic process. Given the above, the present article and research did not need to be discussed by the Ethics Committee. The consent of the Scientific Committee in LabPON Laboratory was also sufficient.

Consent for Publication

We would also like to inform you that the study does not include the patient's personal information. Only statistics on the diagnostic workflow are used. The study compared the time it took to read

and identify key features of tumors using digital (whole slide images) software analysis and the traditional diagnostic method. Accordingly, no applicable consent from the patient and their legal representative.

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