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Considerable interlaboratory variation in PD-L1 positivity in a nationwide cohort of non-small cell lung cancer patients

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ABSTRACT

Objectives: Immunohistochemical expression of programmed death-ligand 1 (PD-L1) is used as a predictive biomarker for prescription of immunotherapy to non-small cell lung cancer (NSCLC) patients. Accurate assessment of PD-L1 expression is therefore crucial. In this study, the extent of interlaboratory variation in PD-L1 positivity in the Netherlands was assessed, using real-world clinical pathology data.

Materials and Methods: Data on all NSCLC patients in the Netherlands with a mention of PD-L1 testing in their pathology report from July 2017 to December 2018 were extracted from PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands. PD-L1 positivity rates were determined for each laboratory that performed PD-L1 testing, with separate analyses for histological and cytological material. Two cutoffs (1% and 50%) were used to determine PD-L1 positivity. Differences between laboratories were assessed using funnel plots with 95% confidence limits around the overall mean.

Results: 6,354 patients from 30 laboratories were included in the analysis of histology data. At the 1% cutoff, maximum interlaboratory variation was 39.1% (32.7%–71.8%) and ten laboratories (33.3%) differed significantly from the mean. Using the 50% cutoff, four laboratories (13.3%) differed significantly from the mean and maximum variation was 23.1% (17.2%–40.3%). In the analysis of cytology data, 1,868 patients from 23 laboratories were included. Eight laboratories (34.8%) differed significantly from the mean in the analyses of both cutoffs. Maximum variation was 41.2% (32.2%–73.4%) and 29.2% (14.7%–43.9%) using the 1% and 50% cutoffs, respectively.

Conclusion: Considerable interlaboratory variation in PD-L1 positivity was observed. Variation was largest using the 1% cutoff. At the 50% cutoff, analysis of cytology data demonstrated a higher degree of variation than the analysis of histology data.

1. Introduction

Therapies targeting programmed death receptor-1 (PD-1) or its ligand programmed death ligand-1 (PD-L1) have become a relevant component of standard treatment regimens in patients with advanced non-small cell lung cancer (NSCLC) [1,2]. In clinical practice, oncologists rely on results from PD-L1 immunohistochemistry (IHC) to make treatment decisions for NSCLC patients. PD-L1 IHC is performed in pathology laboratories and assessed by pathologists, who determine the percentage of tumor cells that show PD-L1 expression relative to the total amount of tumor cells present (tumor proportion score (TPS)). Based on the TPS, patients are offered different treatment options. For instance, in many European countries, the PD-L1 inhibitor durvalumab, prescribed as consolidation treatment to stage III NSCLC patients, is only reimbursed for patients whose tumors show a PD-L1 TPS of $\geq$ 1%. This is based on advice from the European Medicines Agency (EMA) [3].

Abbreviations: CCMO, Central Committee on Research involving Human Subjects; EMA, European Medicines Agency; EQA, external quality assessment; GDPR, General Data Protection Regulation; IHC, immunohistochemistry; LDT, laboratory-developed test; NSCLC, non-small cell lung cancer; PD-1, programmed death receptor-1; PD-L1, programmed death ligand-1; TCs, tumor cells; TPS, tumor proportion score.

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Similarly, only patients with advanced NSCLC whose tumor biopsy expresses a PD-L1 TPS of ≥ 50% are offered the option of monotherapy with the PD-1 inhibitor pembrolizumab, which is significantly better tolerated than the standard combination strategy which includes chemotherapy [1,2]. Therefore, it is crucial that TPS is determined reliably by all pathology laboratories that perform PD-L1 IHC.

An important factor in accurate assessment of PD-L1 TPS is the scoring performed by pathologists. Studies have shown that in a real-world clinical setting, substantial variation exists in histologic grading of various tumor types between laboratories [4–7]. Regarding PD-L1, it has been reported that inter-pathologist agreement of PD-L1 scoring on tumor cells may be high, but is often found to be decreased when using the 1% cutoff [8]. Discordance in PD-L1 scoring between pathologists might result in a high degree of variation in PD-L1 positivity rates between laboratories. Whether this is in fact true for clinical practice, is of yet unknown.

Other analytical and pre-analytical factors of IHC could also contribute to interlaboratory variation in positivity rates of IHC biomarkers [9,10]. With PD-L1 IHC, laboratories may for instance differ in their choice of antibody, as multiple antibodies are available and it is unfeasible for laboratories to use more than one antibody for the same test [10]. Some studies have questioned whether different PD-L1 assays and laboratory-developed tests (LDTs) can be used interchangeably [8,11]. Furthermore, whereas processing of histological material is often performed in a similar way by different laboratories, many differences can be found in processing of cytological material [12–14]. It is possible that these differences might increase interlaboratory variation in PD-L1 positivity.

A high degree of variation in PD-L1 positivity rates between pathology laboratories could result in patients being scored as PD-L1 positive in one laboratory and as PD-L1 negative in another. This could lead to patients being denied effective treatment options or being exposed to unnecessary toxicity. In order to determine whether this may be the case in the real-world setting, we conducted a retrospective cohort study to assess the existence of interlaboratory variation in PD-L1 positivity in clinical practice in the Netherlands, using real-world clinical pathology data of NSCLC patients.

2. Materials and methods

2.1. Data extraction

Data were extracted from a national database governed by the PALGA foundation, the nationwide network and registry of histo- and cytopathology in the Netherlands. This database contains excerpts from pathology reports dating back to 1971 and manages all pathology records from all Dutch pathology laboratories since 1991 [15]. Patients at each individual institution connected to PALGA can opt-out of consenting to the use of their data for research, which is estimated to occur in 3% of all patients [16]. All personal data in the database are pseudonymized by a Trusted Third Party (ZorgTTP, Houten, the Netherlands), ensuring that individual patients are not identifiable. According to the Central Committee on Research involving Human Subjects (CCMO), this type of study does not require approval from an ethics committee in the Netherlands. This study was approved by PALGA's Scientific Council and Privacy Committee and all data was handled according to the General Data Protection Regulation (GDPR).

Data were retrieved on all NSCLC patients in the Netherlands with a mention of PD-L1 testing in their pathology report between 1 July 2017 and 31 December 2018. Patients with two primary lung tumors were excluded, since treatment of the one tumor might have influenced PD-L1 expression on the other tumor [17,18]. For each patient, the following data were extracted: age, sex, histologic subtype, amount of PD-L1 tests performed, source of material for PD-L1 test(s), type of material for PD-L1 test(s), antibody and protocol (commercial assay or LDT) used for PD-L1 test(s), reported TPS and number of tumor cells (TCs) present. Additionally, information on academic or non-academic status was subtracted for each laboratory.

2.2. Analysis of interlaboratory variation in PD-L1 positivity

To study variation in PD-L1 positivity, we compared the percentages of reported PD-L1 positive patients between all laboratories that performed PD-L1 testing. We determined PD-L1 positivity based on two cutoffs for TPS relevant in clinical practice, i.e. ≥ 1% and ≥ 50%. Interlaboratory variation was studied with separate analyses for each of these cutoffs: one analysis with data dichotomized according to the 1% cutoff, and one with data dichotomized according to the 50% cutoff. Moreover, since processing of cytological material may vary greatly between laboratories, we analyzed histological and cytological material separately.

For each patient, only one PD-L1 test was included for analysis. If patients had had PD-L1 testing performed on both histological and cytological material, they were included in both the analysis of histological material and the analysis of cytological material. Only laboratories that performed PD-L1 testing in ≥ 30 patients during the study period were included in the final analyses. If test results of patients with multiple PD-L1 tests performed on the same type of material (either all on histology or all on cytology) were discordant, these patients were excluded. Patients with inconclusive test results only and patients with tests without a reported TPS were excluded as well.

2.3. Analysis based on IHC antibody and protocol

In order to get a preliminary idea of the role that different antibodies might play in causing variation in PD-L1 positivity, separate analyses were performed that incorporated information on use of IHC antibody and protocol (commercial assay or LDT) by individual laboratories in plots displaying interlaboratory variation in PD-L1 positivity rates. These plots were created using histology data only.

2.4. Statistical analysis

Patient and PD-L1 test characteristics were summarized using counts and proportions for histological and cytological material separately. Differences between PD-L1 positive and negative subgroups were tested by using a χ^2-test for categorical variables and a *t*-test for continuous variables.

The overall proportions of PD-L1 positivity were determined for histological and cytological material separately, using both the 1% cutoff and the 50% cutoff. These overall proportions were considered the mean national proportion. Differences in PD-L1 positivity rates between laboratories were assessed by creating funnel plots, which display the mean national proportion of PD-L1 positivity with its 95% confidence limits and the percentage of PD-L1 positive patients plotted against the total number of patients tested for each laboratory. This allows for comparison of laboratories to each other and to the mean [19]. All laboratories falling outside the 95% confidence limits were considered to differ significantly from the mean. Academic and non-academic laboratories were indicated separately within the funnel plots.

The positivity rates displayed in the funnel plots for each individual laboratory were adjusted for case mix (i.e. difference in patient and test characteristics) by performing multivariate logistic regression analysis using predetermined variables. These variables included age, sex, histologic subtype and source of material for PD-L1 testing. For the analysis of histology data, type of material (i.e. biopsy or resection) was also added to the logistic regression model. As information on the IHC antibody and/or protocol used for PD-L1 testing was lacking in a considerable percentage of cases (in 32.3% and 40.3% of cases using histology and cytology data, respectively), this variable was not included in the main multivariate logistic regression model. In order to get a general idea of the role that use of different IHC antibodies and

protocols might play in causing interlaboratory variation in PD-L1 positivity, additional case mix adjusted PD-L1 positivity rates were calculated using multivariate logistic regression analysis that did include the variable IHC antibody/protocol. These positivity rates were then compared with the case mix adjusted positivity rates without the variable IHC antibody/protocol, by displaying both in one funnel plot. Case mix adjusted positivity rates were determined by dividing the observed percentage of PD-L1 positive patients per laboratory by the expected percentage, based on the multivariate logistic regression model, followed by multiplying with the national mean percentage of PD-L1 positivity.

All statistical analysis was performed using IBM SPSS Statistics version 25.

3. Results

3.1. Patient selection process

Data of 9,153 NSCLC patients with a mention of PD-L1 in their pathology report were retrieved from the PALGA database. PD-L1 testing was performed on histological and/or cytological material of 8,725 of these patients, with 10,625 PD-L1 tests performed in total. Data from 42 pathology laboratories were included in the dataset, of which 32 performed PD-L1 testing themselves. PD-L1 testing was performed on histological material in 6,755 cases and on cytological material in 2,300 cases. For the analysis of histology data, two laboratories that performed PD-L1 testing in < 30 patients were excluded, resulting in exclusion of 43 patients. After exclusion of patients with discordant results of multiple PD-L1 tests (n = 178), patients with inconclusive test results only (n = 166) and patients with tests with unknown TPS (n = 14), 6,354 patients from 30 laboratories remained for analysis of interlaboratory variation using data on histological material (Fig. 1). The 166 patients with inconclusive test results only had a total of 177 tests performed. Of these tests, 143 (80.8%) were inconclusive because the amount of viable tumor cells was insufficient (< 100). In the remaining cases, various reasons why the test was considered inconclusive were reported, such as too much background staining, hard to distinguish tumor cells within inflammatory infiltrate, and mechanical damage to tissue. In some cases, the reason why the test was considered inconclusive was not reported. For the analysis of cytology data, patients from nine laboratories that performed PD-L1 testing in < 30 patients were excluded (n = 111). 1,868 patients from 23 laboratories remained for analysis of interlaboratory variation after exclusion of patients with discordant results of multiple PD-L1 tests (n = 23), patients with inconclusive test results only (n = 290) and patients with tests with unknown TPS (n = 8) (Fig. 1). A total of 309 tests was performed in the patients with inconclusive test results only. In 236 (76.4%) of these tests, there was an insufficient amount of viable tumor cells (< 100).

Characteristics of all patients included in the analysis of histology data are displayed in Table 1. Proportions significantly differed between PD-L1 positivity and negativity across histologic subtype, source of material, and type of material for both cutoffs (i.e. 1% and 50%) and across IHC antibody/protocol at the 1% cutoff, although the observed differences in percentages were sometimes small and not always clinically relevant. Table 2 shows the characteristics of all patients included in the analysis of cytology data. Proportions across sex, histologic subtype and IHC antibody/protocol differed significantly between PD-L1 positivity and PD-L1 negativity for both cutoffs. All differences in patient/specimen characteristics between PD-L1 positivity and PD-L1 negativity displayed in Table 1 and Table 2 were corrected for through multivariate logistic regression in our final data analyses, except for the differences in IHC antibody/protocol, since it was unknown which PD-L1 antibody and/or staining protocol was used in a considerable percentage of cases.

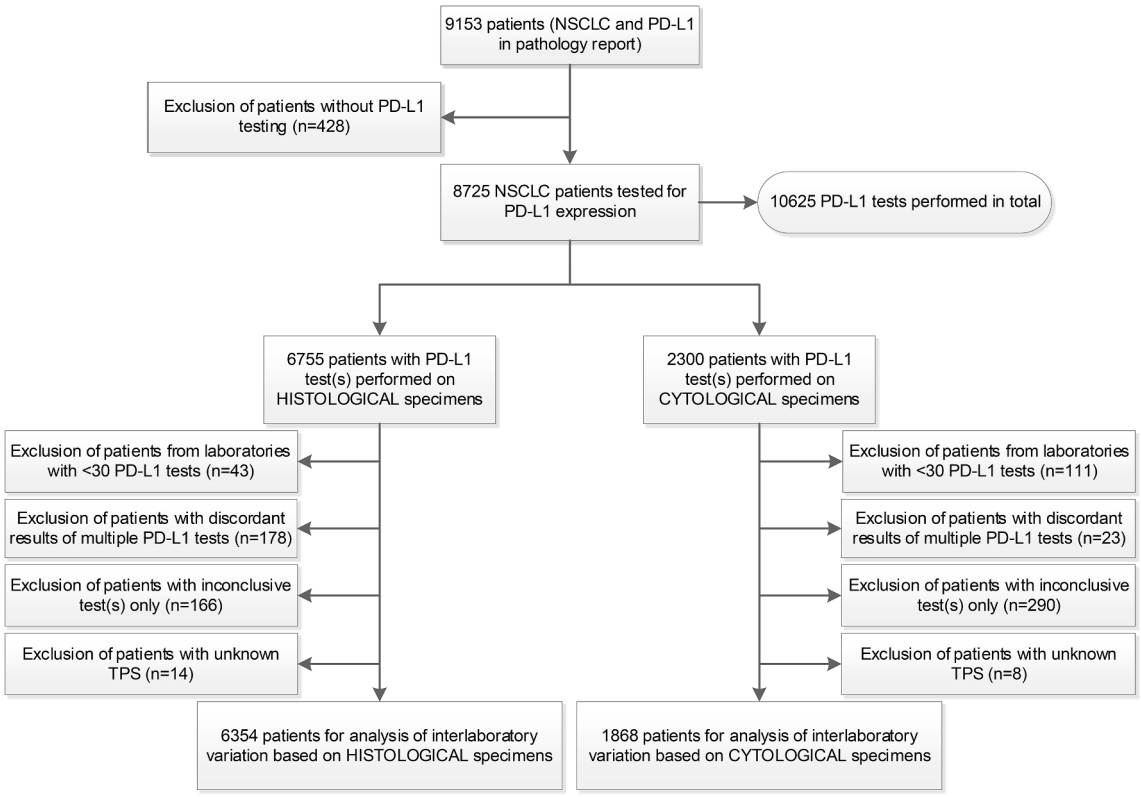

Fig. 1. Flowchart of patient selection process. Abbreviations: NSCLC = non-small cell lung cancer; PD-L1 = programmed death-ligand 1; TPS = tumor proportion score.

Table 1
Characteristics for two sets of groups (PD-L1 < 1% vs. PD-L1 ≥ 1% and PD-L1 < 50% vs. PD-L1 ≥ 50%) using data on histological material only.

	Total (n = 6354)	PD-L1 < 1%(n = 2763)	PD-L1 ≥ 1%(n = 3591)	*p*-value	PD-L1 < 50%(n = 4387)	PD-L1 ≥ 50%(n = 1967)	*p*-value
Age in years (mean (SD))	67.1 (9.6)	67.1 (9.4)	67.1 (9.7)	0.90	67.2 (9.5)	66.9 (9.7)	0.37
Sex							
Male	3498 (55.1%)	1518 (54.9%)	1980 (55.1%)	0.88	2420 (55.2%)	1078 (54.8%)	0.79
Female	2856 (44.9%)	1245 (45.1%)	1611 (44.9%)		1967 (44.8%)	889 (45.2%)	
Histologic subtype							
AC	4139 (65.1%)	1769 (64.0%)	2370 (66.0%)	<0.001	2774 (63.2%)	1365 (69.4%)	<0.001
SCC	1484 (23.4%)	664 (24.0%)	820 (22.8%)		1116 (25.4%)	368 (18.7%)	
NSCLC NOS	504 (7.9%)	196 (7.1%)	308 (8.6%)		322 (7.3%)	182 (9.3%)	
Other	227 (3.6%)	134 (4.8%)	93 (2.6%)		175 (4.0%)	52 (2.6%)	
Source of material							
Primary tumor	3935 (61.9%)	1714 (62.0%)	2221 (61.8%)	<0.001	2769 (63.1%)	1166 (59.3%)	<0.001
Metastasis	1493 (23.5%)	700 (25.3%)	793 (22.1%)		1049 (23.9%)	444 (22.6%)	
Lymph node metastasis	863 (13.6%)	316 (11.4%)	547 (15.2%)		514 (11.7%)	349 (17.7%)	
Other	63 (1.0%)	33 (1.2%)	30 (0.8%)		55 (1.3%)	8 (0.4%)	
Type of material							
Biopsy	5467 (86.0%)	2342 (84.8%)	3125 (87.0%)	0.01	8736 (85.2%)	1731 (88.0%)	<0.01
Surgical resection	887 (14.0%)	421 (15.2%)	466 (13.0%)		651 (14.8%)	236 (12.0%)	
IHC antibody/protocol							0.06
SP263	2084 (32.8%)	882 (31.9%)	1202 (33.5%)	<0.001	1448 (33.0%)	636 (32.3%)	
22C3 LDT	1437 (22.6%)	647 (23.4%)	790 (22.0%)		1010 (23.0%)	427 (21.7%)	
22C3 pharmDx	630 (9.9%)	244 (8.8%)	386 (10.7%)		405 (9.2%)	225 (11.4%)	
22C3 ?	1262 (19.9%)	521 (18.9%)	741 (20.1%)		855 (19.5%)	407 (20.7%)	
Other	155 (2.4%)	76 (2.8%)	79 (2.2%)		110 (2.5%)	45 (2.3%)	
Unknown	786 (12.4%)	393 (14.2%)	393 (10.9%)		559 (12.7%)	227 (11.5%)	

Abbreviations: AC = adenocarcinoma; IHC = immunohistochemistry; LDT = laboratory-developed test; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; PD-L1 = programmed death-ligand 1; SD = standard deviation; SCC = squamous cell carcinoma; ? = IHC protocol unknown.

Table 2
Characteristics for two sets of groups (PD-L1 < 1% vs. PD-L1 ≥ 1% and PD-L1 < 50% vs. PD-L1 ≥ 50%) using data on cytological material only.

	Total(n = 1868)	PD-L1 < 1%(n = 907)	PD-L1 ≥ 1%(n = 961)	*p*-value	PD-L1 < 50%(n = 1330)	PD-L1 ≥ 50%(n = 538)	*p*-value
Age in years (mean (SD))	66.9 (10.1)	66.9 (10.2)	66.8 (10.1)	0.79	66.9 (10.2)	66.7 (10.1)	0.72
Sex							
Male	998 (53.4%)	523 (57.7%)	475 (49.4%)	<0.001	743 (55.9%)	255 (47.4%)	0.001
Female	870 (46.6%)	384 (42.3%)	486 (50.6%)		587 (44.1%)	283 (52.6%)	
Histologic subtype							
AC	1445 (77.4%)	663 (73.1%)	782 (81.4%)	<0.001	996 (74.9%)	449 (83.3%)	0.001
SCC	243 (13.0%)	141 (15.5%)	102 (10.6%)		193 (14.5%)	50 (9.3%)	
NSCLC NOS	160 (8.6%)	89 (9.8%)	71 (7.4%)		125 (9.4%)	35 (6.5%)	
Other	20 (1.1%)	14 (1.5%)	6 (0.6%)		16 (1.2%)	4 (0.7%)	
Source of material							
Primary tumor	100 (5.4%)	50 (5.5%)	50 (5.2%)	0.23	73 (5.5%)	27 (5.0%)	0.19
Metastasis	128 (6.9%)	69 (7.6%)	59 (6.1%)		90 (6.8%)	38 (7.1%)	
Lymph node metastasis	1047 (56.0%)	520 (57.3%)	527 (54.8%)		735 (55.3%)	312 (58.0%)	
Pleural effusion	494 (26.4%)	219 (24.1%)	275 (28.6%)		351 (26.4%)	143 (26.6%)	
Bronchial brush/fluid	99 (5.3%)	49 (5.4%)	50 (5.2%)		81 (6.1%)	18 (3.3%)	
IHC antibody/protocol							
SP263	483 (25.9%)	274 (30.2%)	209 (21.7%)	<0.001	380 (28.6%)	103 (19.1%)	<0.001
22C3 LDT	464 (24.8%)	228 (25.1%)	236 (24.6%)		326 (24.5%)	138 (25.7%)	
22C3 pharmDx	104 (5.6%)	47 (5.2%)	57 (5.9%)		75 (5.6%)	29 (5.4%)	
22C3 ?	372 (19.9%)	146 (16.1%)	226 (23.5%)		243 (18.3%)	129 (24.0%)	
Other	63 (3.4%)	21 (2.3%)	42 (4.4%)		38 (2.9%)	25 (4.6%)	
Unknown	382 (20.4%)	191 (21.1%)	191 (19.9%)		268 (20.2%)	114 (21.2%)	

Abbreviations: AC = adenocarcinoma; IHC = immunohistochemistry; LDT = laboratory-developed test; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; PD-L1 = programmed death-ligand 1; SD = standard deviation; SCC = squamous cell carcinoma; ? = IHC protocol unknown.

3.2. Interlaboratory variation in PD-L1 positivity: Histology

Thirty laboratories performed PD-L1 testing on histological material of ≥ 30 patients. Using the 1% cutoff to determine PD-L1 positivity, the mean national proportion of PD-L1 positive patients was 56.5%. The case mix adjusted positivity rates of the individual laboratories ranged from 32.7% to 71.8%, resulting in a maximum variation of 39.1% between laboratories. Ten (33.3%) laboratories differed significantly from the mean (Fig. 2a).

When the 50% cutoff was used to determine PD-L1 positivity, the mean national proportion of PD-L1 positive patients was 31.0%. With this cutoff, the case mix adjusted positivity rates of the individual laboratories ranged from 17.2% to 40.3%, resulting in a maximum variation of 23.1%. Four (13.3%) laboratories differed significantly from the mean (Fig. 2b).

When comparing academic and non-academic laboratories in the funnel plots of both cutoffs, there did not appear to be any obvious clustering of academic or non-academic laboratories on one side of the national mean. Of the ten laboratories that differed significantly from the mean at the 1% cutoff, four were academic. In the analysis of the 50% cutoff, two of four laboratories that differed significantly from the mean were academic laboratories.

3.3. Interlaboratory variation in PD-L1 positivity: Cytology

Twenty-three laboratories performed PD-L1 testing on cytological

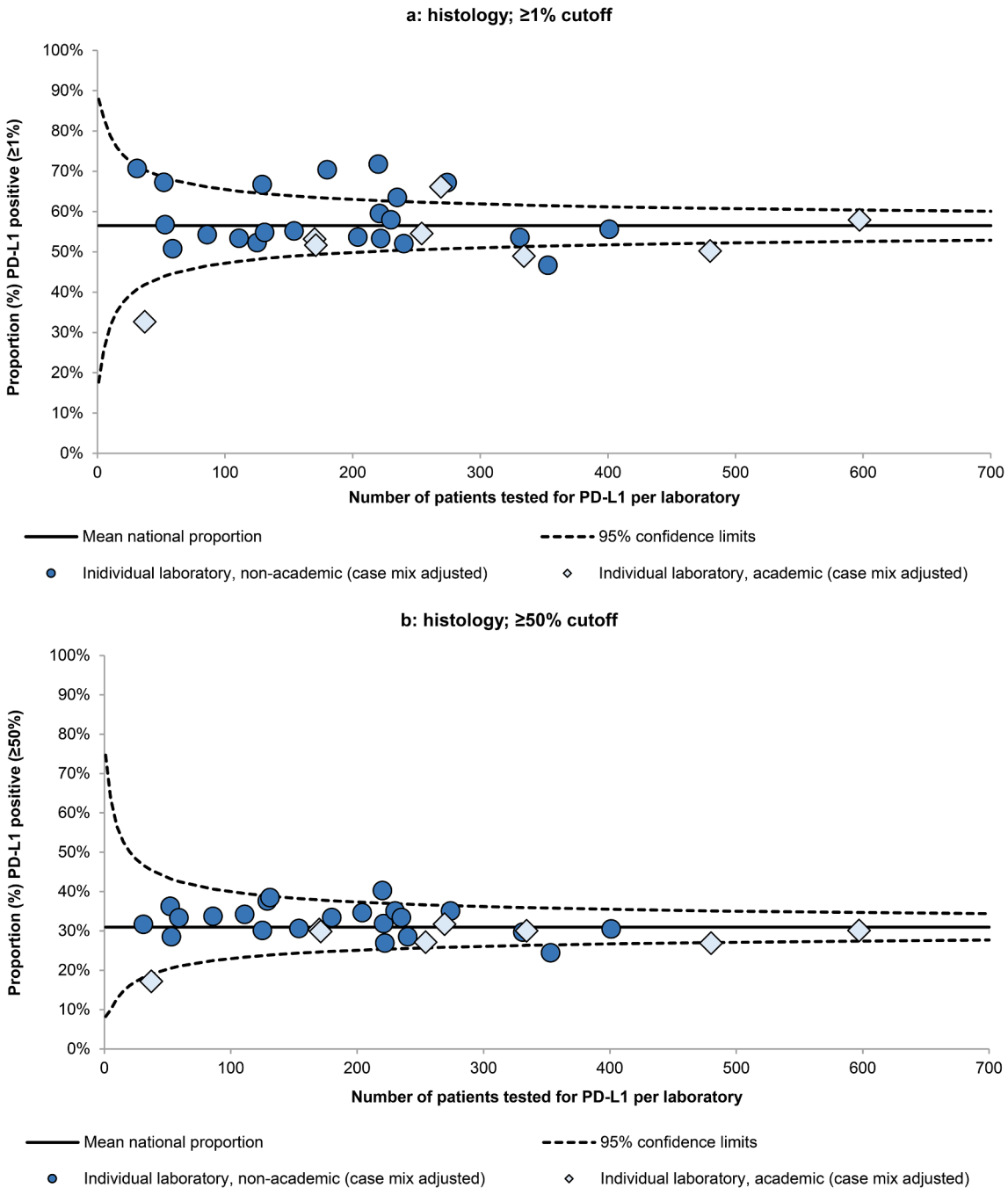

Fig. 2. Funnel plots showing interlaboratory variation in PD-L1 positivity, based on histology data and using either a 1% cutoff (**a**) or a 50% cutoff (**b**) to determine PD-L1 positivity. Case mix adjusted positivity rates are displayed for each laboratory, plotted against the total number of patients tested for PD-L1. The black line shows the mean national proportion of PD-L1 positive patients, surrounded by its 95% confidence limits (dotted lines).

material of ≥ 30 patients. When the 1% cutoff was used to determine PD-L1 positivity, the mean national proportion of PD-L1 positive patients was 51.4%. Individual laboratory case mix adjusted positivity rates ranged from 32.2% to 73.4%, resulting in a maximum variation of 41.2%. Eight (34.8%) laboratories differed significantly from the mean (Fig. 3a).

Using the 50% cutoff to determine PD-L1 positivity, the mean national proportion of PD-L1 positive patients was 28.8%. This time, case mix adjusted positivity rates of individual laboratories ranged from 14.7% to 43.9%, resulting in a maximum variation of 29.2%. Again, eight (34.8%) laboratories differed significantly from the mean (Fig. 3b).

A comparison of academic and non-academic laboratories in the funnel plots of both cutoffs showed no obvious clustering of academic or non-academic laboratories on one side of the national mean. Of the eight laboratories that differed significantly from the mean at the 1% cutoff, three were academic. In the analysis of the 50% cutoff, four of eight laboratories that differed significantly from the mean were academic laboratories.

3.4. Comparing laboratories that differ significantly from the mean

All 23 laboratories that were included in the analysis of cytology data, were also included in the analysis of histology data. When using the 1% cutoff to determine PD-L1 positivity, only two of these 23 laboratories differed significantly from the mean in both the analysis of

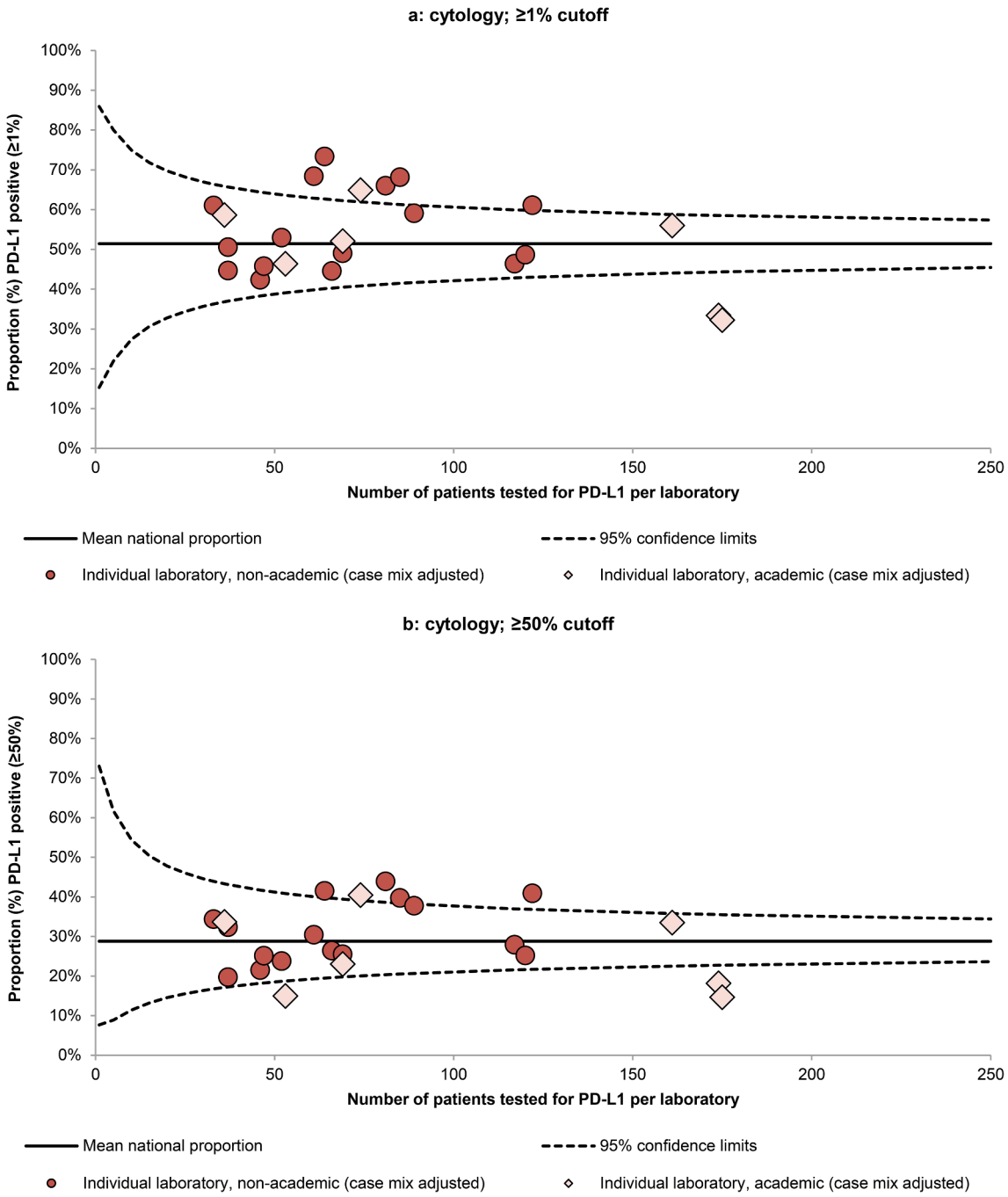

Fig. 3. Funnel plots showing interlaboratory variation in PD-L1 positivity, based on cytology data and using either a 1% cutoff (**a**) or a 50% cutoff (**b**) to determine PD-L1 positivity. Case mix adjusted positivity rates are displayed for each laboratory, plotted against the total number of patients tested for PD-L1. The black line shows the mean national proportion of PD-L1 positive patients, surrounded by its 95% confidence limits (dotted lines).

histology data and the analysis of cytology data. Using the 50% cutoff, only one laboratory differed significantly from the mean in both analyses. Seven laboratories were included in the analysis of histology data only. Three of these seven laboratories differed significantly from the mean in the analysis of the 1% cutoff, and one differed significantly from the mean in the analysis of the 50% cutoff.

3.5. PD-L1 IHC antibodies and protocols

In order to analyze the role that use of different PD-L1 IHC antibodies and protocols (commercial assay or LDT) might play in causing interlaboratory variation in PD-L1 positivity, information on PD-L1 IHC antibodies and protocols used by individual laboratories was incorporated in the funnel plots for histological material (see Supplementary Figure 1a and b). There did not appear to be any obvious clustering of specific IHC antibodies on one side of the national mean and the laboratories that differed significantly from the mean used various antibodies. Additionally, no obvious differences were observed between commercial assays and LDTs.

PD-L1 positivity rates that were adjusted for case mix with inclusion of the variable IHC antibody/protocol ranged from 33.2% to 68.4% at the 1% cutoff, resulting in a maximum variation of 35.2% between laboratories. This range was smaller than the range in PD-L1 positivity rates adjusted for case mix without IHC antibody/protocol (maximum

variation 35.2% vs. 39.1%). With the inclusion of IHC antibody/protocol in case mix adjustment, nine (30.0%) laboratories differed significantly from the mean, which is one less than in the analysis excluding IHC antibody/protocol from case mix adjustment. From the laboratories with known PD-L1 antibody and protocol, inclusion of IHC antibody/protocol in case mix adjustment showed the largest effect on laboratories that used the commercial 22C3 pharmDx assay (Supplementary Figure 2a).

At the 50% cutoff, PD-L1 positivity rates adjusted for case mix with inclusion of the variable IHC antibody/protocol ranged from 17.3% to 38.4%, resulting in a maximum variation of 21.1%. There was slightly less variation compared to the analysis of PD-L1 positivity rates adjusted for case mix not including IHC antibody/protocol (maximum variation 21.1% vs. 23.1%). With the inclusion of IHC antibody/protocol in case mix adjustment, only two (6.7%) laboratories differed significantly from the mean, which was two less than in the analysis excluding IHC antibody/protocol from case mix adjustment. Similar to the analysis at the 1% cutoff, inclusion of IHC antibody/protocol in case mix adjustment showed the largest effect on laboratories that used the commercial 22C3 pharmDx assay (Supplementary Figure 2b).

4. Discussion

In this nationwide cohort of NSCLC patients, using real-world clinical pathology data, a considerable amount of variation in PD-L1 positivity was found between laboratories. The amount of variation was largest when a 1% cutoff was used to determine PD-L1 positivity. When using a 50% cutoff, use of cytological material for PD-L1 testing also resulted in a substantial amount of variation. Use of the 50% cutoff to determine PD-L1 positivity on histological material resulted in the smallest amount of interlaboratory variation in PD-L1 positivity.

With regard to the analysis of histology data, the difference in amount of variation between the 1% and the 50% cutoff is prominent. The degree of variation seen at the 50% cutoff is a lot smaller compared to the 1% cutoff. Most likely, the higher amount of variation seen when using the 1% cutoff is caused to a large extent by a higher degree of interpathologist variation at this cutoff compared to the 50% cutoff. Several studies have, in fact, demonstrated lower concordance levels of PD-L1 scoring on TCs between pathologists at the 1% cutoff in comparison to the 50% cutoff [20–26]. Apparently, determining PD-L1 positivity using a 1% cutoff is harder than determining PD-L1 positivity at the 50% cutoff level. When some pathologists have a tendency to score dubious cases with a TPS that lies around 1% as PD-L1 positive, while other pathologists are more conservative in their PD-L1 scoring, this could easily lead to a high degree of variation in PD-L1 positivity rates between pathology laboratories. Subsequently, this would influence treatment decisions and possibly outcome for individual NSCLC patients.

It is remarkable that PD-L1 testing was performed on cytological material in a considerable amount of cases, even though PD-L1 immunostaining on cytological specimens was not validated in clinical trials and only a limited amount of studies had assessed histologic-cytologic correlation of PD-L1 immunohistochemistry during our study period [26–29]. Interestingly, use of cytological material not only resulted in a substantial amount of interlaboratory variation in PD-L1 positivity at the 1% cutoff, but also at the 50% cutoff. Moreover, most of the laboratories differing significantly from the mean in analysis of cytology data were different laboratories than the ones that differed significantly from the mean in analysis of histology data. This suggests that, besides interpathologist variation in scoring of PD-L1 TPS, use of cytological material may also contribute to interlaboratory variation in PD-L1 positivity. We hypothesize that this is most likely a result of the previously mentioned differences in processing of cytology [12–14]. This may include the use of fixatives other than formalin, such as alcohol-based fixatives, which have been shown to negatively influence immunoreactivity of various IHC antibodies [30–34]. Use of CytoLyt, a methanol-based fixative, has been shown to negatively affect PD-L1 immunostaining [35]. When laboratories use this fixative on cytological material, this might result in a PD-L1 positivity rate that is substantially lower than that of laboratories that use formalin fixation, for instance. Unfortunately, data extracted from the PALGA database do not contain any information about processing of cytological specimens. Analysis of interlaboratory variation in PD-L1 positivity rate in relation to different methods of processing of cytology of each laboratory, could create more insight into the influence of different processing methods on PD-L1 variation.

Even though the processing of histology samples often shows more similarities between different laboratories than the processing of cytology samples, it is still imaginable that differences in pre-analytical variables in the acquisition and processing of histological material could contribute to interlaboratory variation in PD-L1 positivity. It has been suggested, for instance, that biopsy size and the number of biopsies taken can influence PD-L1 results, with small samples more often resulting in an underestimation of PD-L1 expression [36]. This could potentially lead to variation in PD-L1 positivity between laboratories when some laboratories use smaller core biopsy needles and/or a smaller number of biopsies in their standard biopsy procedures compared to other laboratories.

An analytical factor that might play a role in causing interlaboratory variation in PD-L1 positivity could be the use of different PD-L1 antibodies and protocols within the various laboratories. In our study, funnel plots incorporating information on PD-L1 antibody and protocol per laboratory showed no clustering of specific antibodies or protocols or use of one specific antibody by the laboratories that differed significantly from the mean, which seems promising. Nevertheless, inclusion of the variable IHC antibody/protocol in case mix adjustment resulted in a reduction of maximum variation in PD-L1 positivity between laboratories compared to the analysis that did not include IHC antibody/protocol in case mix adjustment at both cutoffs, although the reduction in maximum variation was small. Adding IHC antibody/protocol to case mix adjustment also resulted in a reduction of the number of laboratories that differed significantly from the mean at both cutoffs. This suggests that use of different PD-L1 antibodies and protocols might have some influence on interlaboratory variation in PD-L1 positivity. It is important to interpret these analyses with caution, since information on specific PD-L1 antibody and protocol or on protocol only was lacking in more than a quarter of laboratories. One study that also used real-world data on PD-L1 testing [37], showed no statistically significant difference in PD-L1 expression between commercial assays 22C3 and 28–8. Yet, the researchers did find a statistically significant difference at the 50% cutoff level between these two commercial assays and all LDTs grouped together, with the LDTs showing more PD-L1 negative results. Another study showed substantial interlaboratory concordance of PD-L1 staining for various commercial assays, but only moderate concordance for LDTs compared to commercial assays [38]. In contrast, a *meta*-analysis of diagnostic accuracy of PD-L1 IHC assays concluded that properly designed LDTs may in fact achieve higher accuracy than commercial PD-L1 assays, when both are compared to an appropriate reference standard [11]. Various other studies have shown substantial interlaboratory concordance of PD-L1 staining for several commercial PD-L1 assays [22,39–41], while another study stated that equivalence of commercial PD-L1 assays at the 1% and 50% cutoff cannot be assumed [42]. Lastly, a study by Butter et al. [43] showed a similar degree of interlaboratory concordance between laboratories using a 22C3 LDT and laboratories using the 22C3 pharmDx commercial assay (Agilent), but also concluded that interlaboratory variability of immunostaining contributes to discrepancies in PD-L1 positivity between centers. Unfortunately, based on the data in our study, no definitive conclusions can be drawn regarding the actual influence of different antibodies and protocols on interlaboratory variation in PD-L1 positivity in the real life clinical setting.

A large degree of variation between laboratories, such as seen in our study, is problematic. After all, this implies that a patient could receive

different PD-L1 test results depending on the pathology laboratory where his or her material is tested. In turn, this could result in different courses of treatment, and may subsequently influence outcome of individual NSCLC patients. Variation at the 50% cutoff may be especially problematic, since this cutoff is used across the world to differentiate between treatment with immunotherapy alone or a more toxic treatment regimen of immunotherapy combined with chemotherapy for patients with metastatic NSCLC [1,44,45]. With this study, we hope to raise awareness among pathologists, but also among pulmonologists, of the existence of interlaboratory variation in PD-L1 positivity. In order to further decrease variation between laboratories, one could think of various measures pathologists could take, such as double reading of difficult cases. Also, laboratories might decide to let all PD-L1 staining be scored by properly trained and experienced pathologists only, since it has been shown that training for PD-L1 scoring and experience in routine pathology practice correlate with higher inter-pathologists concordance [46]. Unfortunately, our data do not include information about individual laboratories' expertise in scoring PD-L1 on material from NSCLC patients, hence we cannot draw conclusions on the correlation between laboratories' level of experience and the amount of interlaboratory variation in PD-L1. In future, digital image analysis for PD-L1 scoring might improve inter-observer concordance [47], although this needs to be studied more extensively before implementation into clinical practice. Parallel to the previously mentioned measures, which mainly focused on reducing inter-observer variability, a reduction of technical differences between laboratories might also contribute to a decrease in interlaboratory variation. We do not know how many of the laboratories included in our study participated in PD-L1 external quality assessment (EQA) schemes during the study period. Potentially, participation of all laboratories in such EQA schemes could help in reducing interlaboratory variation in PD-L1 positivity [48]. Also, when LDTs are used for PD-L1 staining, it is paramount that these LDTs are optimized and validated sufficiently [49]. This may not always be the case in all laboratories, which may suggest that use of commercial assays could also contribute to reduction of interlaboratory variability in PD-L1 positivity. Nevertheless, this remains uncertain, since even laboratories that use the same commercial assay can produce differences in PD-L1 staining results [38,43] and others have reported inequality of commercial assays at the 1% and 50% cutoff [42]. To help create more awareness among pathologists, results from individual laboratories in our study were sent back to these laboratories as feedback reports. In this way, pathologists are encouraged to discuss and reflect on their own results concerning PD-L1 testing, compared to other laboratories in the Netherlands, and to think of ways to improve their own PD-L1 testing practices.

Our study has some limitations. Notably, some variables were unknown in a large amount of patients, which restricted the analyses that we could perform. This included antibody or specific IHC protocol (commercial assay or LDT) used for PD-L1 testing, as discussed above. Variables such as mutational status, smoking status, and stage of disease were unknown in many or all patients, and could therefore not be included in the multivariate logistic regression model for case mix correction either. It would have strengthened our study if we could have included these variables in our logistic regression model. However, the association between mutation status, such as KRAS and EGFR mutation status, is still controversial, with various studies showing opposing results [50–52]. Also, while some studies show a significant correlation between smoking status and PD-L1 [53], others do not [54,55]. With regard to stage of disease, various studies have shown a positive correlation between higher stages of disease and high PD-L1 expression [53,54]. Since PD-L1 expression holds clinical implications for stage III and stage IV NSCLC patients, most of the patients in our data set would have most likely had stage III or stage IV disease. It is thus unlikely that interlaboratory differences in mutation status, smoking status, or stage of disease could have completely explained the high degree of variation in PD-L1 positivity that we found. Another limitation is the lack of information on the number of pathologists scoring PD-L1 expression per laboratory. While this information could have provided valuable insight into the amount of inter-pathologist variation in PD-L1 positivity rate within each laboratory, we still feel that the insight into the variation in PD-L1 positivity on an interlaboratory level is valuable on its own. To our knowledge, no other studies have provided these kind of analyses on such a large scale using real-world clinical pathology data. Finally, the influence of different methods of processing of cytology material on interlaboratory variation in PD-L1 positivity could not be studied, as information on processing of cytological specimens was not part of the PALGA database. We intend to retrieve this information in another way, such as through questionnaires sent out to laboratories, so that we will be able to study the relationship between processing of cytological specimens and interlaboratory variation in PD-L1 positivity in the future.

In conclusion, we have shown that in a real-world setting, a considerable amount of variation in PD-L1 positivity rates exists between pathology laboratories on a nationwide level. Most likely, this is caused to a large extent by discordance between pathologists at the 1% cutoff point. Potentially, various analytical or pre-analytical factors, such as differences in processing of cytological material between laboratories, may contribute to interlaboratory variation in PD-L1 positivity as well. It is important that the amount of variation between laboratories is reduced, since a high degree of variation could result in patients receiving a different course of treatment when PD-L1 is assessed in the one laboratory compared to another. Both pathologists and pulmonologists should be made aware of this risk, and work together to try to reduce the amount of interlaboratory variation in PD-L1 positivity.

CRediT authorship contribution statement

Bregje M. Koomen: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Quirinus J.M. Voorham:** Resources, Data curation, Writing - review & editing. **Chantal C.H.J. Epskamp-Kuijpers:** Conceptualization, Methodology, Writing - review & editing. **Carmen van Dooijeweert:** Methodology, Writing - review & editing. **Anne S.R. van Lindert:** Conceptualization, Writing - review & editing. **Ivette A.G. Deckers:** Methodology, Writing - review & editing. **Stefan M. Willems:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

SM Willems received research grants from Amgen, AstraZeneca, Bayer, BMS, MSD, NextCure, Pfizer and Roche, all outside the submitted work. ASR van Lindert reports consultation fees (paid to the institution) from AstraZeneca and Roche, outside the submitted work. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] D. Planchard, S. Popat, K. Kerr, S. Novello, E.F. Smit, C. Faivre-Finn, T.S. Mok, M. Reck, P.E. Van Schil, M.D. Hellmann, S. Peters, Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 29 (2018) iv192–iv237, https://doi.org/10.1093/annonc/mdy275.

[2] European Society for Medical Oncology (ESMO). Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, 2019. Available from: https://www.esmo.org/Guidelines/Lung-and-Chest-Tumours/Metastatic-Non-Small-Cell-Lung-Cancer. Accessed 25 November 2019.

[3] EMA. Imfinzi (Durvalumab), 2018. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/imfinzi. Accessed 25 November 2019.

[4] C.C. Kuijpers, C.E. Sluijter, J.H. von der Thusen, et al. Interlaboratory variability in the histologic grading of colorectal adenocarcinomas in a nationwide cohort, Am. J. Surg. Pathol. 40(8) 2016 1100-1108. DOI: 10.1097/PAS.0000000000000636.

[5] C.C. Kuijpers, C.E. Sluijter, J.H. von der Thüsen, K. Grünberg, M.G.H. van Oijen, P. J. van Diest, M. Jiwa, I.D. Nagtegaal, L.I.H. Overbeek, S.M. Willems, Interlaboratory variability in the grading of dysplasia in a nationwide cohort of colorectal adenomas, Histopathology 69 (2) (2016) 187–197, https://doi.org/10.1111/his.2016.69.issue-210.1111/his.12923.

[6] C. van Dooijeweert, P.J. van Diest, S.M. Willems, C. Kuijpers, L.I.H. Overbeek, I.A. G. Deckers, Significant inter- and intra-laboratory variation in grading of ductal carcinoma in situ of the breast: a nationwide study of 4901 patients in the Netherlands, Breast Cancer Res Treat. 174 (2) (2019) 479–488, https://doi.org/10.1007/s10549-018-05082-y.

[7] C. van Dooijeweert, P.J. van Diest, S.M. Willems, et al. Significant inter- and intra-laboratory variation in grading of invasive breast cancer: a nationwide study of 33,043 patients in the Netherlands, Int, J, Cancer 2019. DOI: 10.1002/ijc.32330.

[8] B.M. Koomen, S.K. Badrising, M.M. Heuvel, S.M. Willems, Comparability of PD-L1 immunohistochemistry assays for non-small-cell lung cancer: a systematic review, Histopathology 76 (6) (2020) 793–802, https://doi.org/10.1111/his.v76.610.1111/his.14040.

[9] C. van Dooijeweert, I.A.G. Deckers, I.O. Baas, E. van der Wall, P.J. van Diest, Hormone- and HER2-receptor assessment in 33,046 breast cancer patients: a nationwide comparison of positivity rates between pathology laboratories in the Netherlands, Breast Cancer Res. Treat. 175 (2) (2019) 487–497, https://doi.org/10.1007/s10549-019-05180-5.

[10] K.M. Kerr, F.R. Hirsch, Programmed death ligand-1 immunohistochemistry: friend or Foe? Arch. Pathol. Lab. Med. 140 (4) (2016) 326–331, https://doi.org/10.5858/arpa.2015-0522-SA.

[11] E. Torlakovic, H.J. Lim, J. Adam, et al. "Interchangeability" of PD-L1 immunohistochemistry assays: a meta-analysis of diagnostic accuracy, Mod Pathol. 2019. DOI: 10.1038/s41379-019-0327-4.

[12] E. Vigliar, U. Malapelle, A. Iaccarino, G. Acanfora, P. Pisapia, E. Clery, C. De Luca, C. Bellevicine, G. Troncone, PD-L1 expression on routine samples of non-small cell lung cancer: results and critical issues from a 1-year experience of a centralised laboratory, J. Clin. Pathol. 72 (6) (2019) 412–417, https://doi.org/10.1136/jclinpath-2019-205732.

[13] A. Nambirajan, D. Jain, Cell blocks in cytopathology: an update, Cytopathology 29 (6) (2018) 505–524, https://doi.org/10.1111/cyt.2018.29.issue-610.1111/cyt.12627.

[14] D. Jain, S.R. Mathur, V.K. Iyer, Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies, Cytopathology. 25 (6) (2014) 356–371, https://doi.org/10.1111/cyt.12174.

[15] M. Casparie, A.T.M.G. Tiebosch, G. Burger, H. Blauwgeers, A. van de Pol, J.H.J. M. van Krieken, G.A. Meijer, Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive, Cell Oncol. 29 (1) (2007) 19–24, https://doi.org/10.1155/2007/971816.

[16] Netherlands Patients Federation. Rapport Delen van uw data [in Dutch]. 2019. Available from: https://www.patientenfederatie.nl/downloads/rapporten/154-rapport-delen-van-je-data/file. Accessed 12 November 2020.

[17] J. Sheng, W. Fang, J. Yu, N. Chen, J. Zhan, Y. Ma, Y. Yang, Y. Huang, H. Zhao, L. Zhang, Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer, Sci. Rep. 6 (1) (2016), https://doi.org/10.1038/srep20090.

[18] L. Rojkó, L. Reiniger, V. Téglási, K. Fábián, O. Pipek, A. Vágvölgyi, L. Agócs, J. Fillinger, Z. Kajdácsi, J. Tímár, B. Döme, Z. Szállási, J. Moldvay, Chemotherapy treatment is associated with altered PD-L1 expression in lung cancer patients, J. Cancer Res. Clin. Oncol. 144 (7) (2018) 1219–1226, https://doi.org/10.1007/s00432-018-2642-4.

[19] D.J. Spiegelhalter. Funnel plots for comparing institutional performance. Stat Med. 24(8) 2005 1185-1202. DOI: 10.1002/sim.1970.

[20] A.W.H. Chan, J.H.M. Tong, J.S.H. Kwan, et al. Assessment of programmed cell death ligand-1 expression by 4 diagnostic assays and its clinicopathological correlation in a large cohort of surgical resected non-small cell lung carcinoma, Mod Pathol. 2018. DOI: 10.1038/s41379-018-0053-3.

[21] H. Brunnström, A. Johansson, S. Westbom-Fremer, M. Backman, D. Djureinovic, A. Patthey, M. Isaksson-Mettävainio, M. Gulyas, P. Micke, PD-L1 immunohistochemistry in clinical diagnostics of lung cancer: Inter-pathologist variability is higher than assay variability, Mod. Pathol. 30 (10) (2017) 1411–1421, https://doi.org/10.1038/modpathol.2017.59.

[22] A. Marchetti, M. Barberis, R. Franco, G. De Luca, M.V. Pace, S. Staibano, M. Volante, F. Buttitta, E. Guerini-Rocco, L. Righi, T. D'antuono, G.V. Scagliotti, C. Pinto, G. De Rosa, M. Papotti, Multicenter comparison of 22C3 PharmDx (Agilent) and SP263 (Ventana) assays to test PD-L1 expression for NSCLC patients to be treated with immune checkpoint inhibitors, J. Thorac. Oncol. 12 (11) (2017) 1654–1663, https://doi.org/10.1016/j.jtho.2017.07.031.

[23] M.J. Ratcliffe, A. Sharpe, A. Midha, C. Barker, M. Scott, P. Scorer, H. Al-Masri, M. C. Rebelatto, J. Walker, Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cutoffs in non-small cell lung cancer, Clin. Cancer Res. 23 (14) (2017) 3585–3591, https://doi.org/10.1158/1078-0432.CCR-16-2375.

[24] D.L. Rimm, G. Han, J.M. Taube, E.S. Yi, J.A. Bridge, D.B. Flieder, R. Homer, W. W. West, H. Wu, A.C. Roden, J. Fujimoto, H. Yu, R. Anders, A. Kowalewski, C. Rivard, J. Rehman, C. Batenchuk, V. Burns, F.R. Hirsch, I.I. Wistuba, A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer, JAMA Oncol. 3 (8) (2017) 1051, https://doi.org/10.1001/jamaoncol.2017.0013.

[25] M.S. Tsao, K.M. Kerr, M. Kockx, M. Beasley, A.C. Borczuk, J. Botling, L. Bubendorf, L. Chirieac, G. Chen, T.-Y. Chou, J.-H. Chung, S. Dacic, S. Lantuejoul, M. Mino-Kenudson, A.L. Moreira, A.G. Nicholson, M. Noguchi, G. Pelosi, C. Poleri, P. A. Russell, J. Sauter, E. Thunnissen, I. Wistuba, H. Yu, M.W. Wynes, M. Pintilie, Y. Yatabe, F.R. Hirsch, PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project, J. Thorac. Oncol. 13 (9) (2018) 1302–1311, https://doi.org/10.1016/j.jtho.2018.05.013.

[26] E. Russell-Goldman, S. Kravets, S.E. Dahlberg, L.M. Sholl, M. Vivero, Cytologic-histologic correlation of programmed death-ligand 1 immunohistochemistry in lung carcinomas, Cancer Cytopathol. 126 (4) (2018) 253–263, https://doi.org/10.1002/cncy.v126.410.1002/cncy.21973.

[27] B.G. Skov, T. Skov, Paired comparison of PD-L1 expression on Cytologic and histologic specimens from malignancies in the lung assessed with PD-L1 IHC 28–8pharmDx and PD-L1 IHC 22C3pharmDx, Appl. Immunohistochem. Mol. Morphol. 25 (7) (2017) 453–459, https://doi.org/10.1097/PAI.0000000000000540.

[28] R. Sakakibara, K. Inamura, Y. Tambo, et al., EBUS-TBNA as a promising Method for the Evaluation of Tumor PD-L1 expression in lung cancer, Clin. Lung Cancer 18 (5) (2017) 527–534.e1, https://doi.org/10.1016/j.cllc.2016.12.002.

[29] M. Ilie, J. Juco, L. Huang, V. Hofman, S. Khambata-Ford, P. Hofman, Use of the 22C3 anti-programmed death ligand 1 antibody to determine programmed death ligand 1 expression in cytology samples obtained from non-small cell lung cancer patients, Cancer Cytopathol. 126 (4) (2018) 264–274, https://doi.org/10.1002/cncy.v126.410.1002/cncy.21977.

[30] D.J. Buonocore, F. Konno, A.A. Jungbluth, et al., CytoLyt fixation significantly inhibits MIB1 immunoreactivity whereas alternative Ki-67 clone 30–9 is not susceptible to the inhibition: Critical diagnostic implications, Cancer Cytopathol. 127 (10) (2019) 643–649, https://doi.org/10.1002/cncy.v127.1010.1002/cncy.22170.

[31] B.K. Gorman, O. Kosarac, S. Chakraborty, M.R. Schwartz, D.R. Mody, Comparison of breast carcinoma prognostic/predictive biomarkers on cell blocks obtained by various methods: Cellient, formalin and thrombin, Acta Cytol. 56 (3) (2012) 289–296, https://doi.org/10.1159/000337436.

[32] J.R. Gruchy, P.J. Barnes, K.A. Dakin Hache, CytoLyt(R) fixation and decalcification pretreatments alter antigenicity in normal tissues compared with standard formalin fixation, Appl. Immunohistochem. Mol. Morphol. 23 (4) (2015) 297–302, https://doi.org/10.1097/pai.0000000000000082.

[33] K.Z. Hanley, G.G. Birdsong, C. Cohen, M.T. Siddiqui, Immunohistochemical detection of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression in breast carcinomas: comparison on cell block, needle-core, and tissue block preparations, Cancer 117 (4) (2009) 279–288, https://doi.org/10.1002/cncy.v117:410.1002/cncy.20034.

[34] J.L. Sauter, K.L. Grogg, J.A. Vrana, M.E. Law, J.L. Halvorson, M.R. Henry, Young investigator challenge: validation and optimization of immunohistochemistry protocols for use on cellient cell block specimens, Cancer Cytopathol. 124 (2) (2016) 89–100, https://doi.org/10.1002/cncy.v124.210.1002/cncy.21660.

[35] I.E. Lloyd, W. Zhou, B.L. Witt, B.E. Chadwick, Characterization of PD-L1 immunohistochemical expression in cell blocks with different specimen fixation and processing methods, Appl. Immunohistochem. Mol. Morphol. 27 (2) (2019) 107–113, https://doi.org/10.1097/PAI.0000000000000572.

[36] E. Thunnissen, K.M. Kerr, U. Dafni, et al., Programmed death-ligand 1 expression influenced by tissue sample size. Scoring based on tissue microarrays' and cross-validation with resections, in patients with, stage I-III, non-small cell lung carcinoma of the European Thoracic Oncology Platform Lungscape cohort, Mod. Pathol. 33 (5) (2020) 792–801, https://doi.org/10.1038/s41379-019-0383-9.

[37] V. Velcheti, P.D. Patwardhan, F.X. Liu, X. Chen, X. Cao, T. Burke, Real-world PD-L1 testing and distribution of PD-L1 tumor expression by immunohistochemistry assay type among patients with metastatic non-small cell lung cancer in the United States, PLoS One 13 (11) (2018) e0206370, https://doi.org/10.1371/journal.pone.0206370.

[38] A.H. Scheel, G. Baenfer, G. Baretton, et al., Interlaboratory concordance of PD-L1 immunohistochemistry for non-small-cell lung cancer, Histopathology 72 (3) (2018) 449–459, https://doi.org/10.1111/his.2018.72.issue-310.1111/his.13375.

[39] J. Adam, N. Le Stang, I. Rouquette, A. Cazes, C. Badoual, H. Pinot-Roussel, L. Tixier, C. Danel, F. Damiola, D. Damotte, F. Penault-Llorca, S. Lantuéjoul, Multicenter harmonization study for PD-L1 IHC testing in non-small-cell lung

cancer, Ann. Oncol. 29 (4) (2018) 953–958, https://doi.org/10.1093/annonc/mdy014.
[40] C. Roach, N. Zhang, E. Corigliano, et al. Development of a companion diagnostic PD-L1 immunohistochemistry assay for Pembrolizumab therapy in non-small-cell lung cancer, Appl. Immunohistochem. Mol. Morphol. 24(6) 2016 392-397. DOI: 10.1097/PAI.0000000000000408.
[41] B. Vennapusa, B. Baker, M. Kowanetz, et al. Development of a PD-L1 complementary diagnostic immunohistochemistry assay (SP142) for Atezolizumab, Appl. Immunohistochem. Mol. Morphol. 27(2) 2019 92-100. DOI: 10.1097/PAI.0000000000000594.
[42] A. Dodson, S. Parry, B. Lissenberg-Witte, et al., External quality assessment demonstrates that PD-L1 22C3 and SP263 assays are systematically different, J. Pathol. Clin. Res. 6 (2) (2020) 138–145, https://doi.org/10.1002/cjp2.v6.210.1002/cjp2.153.
[43] R. Butter, N.A. 't Hart, G.K.J. Hooijer, et al., Multicentre study on the consistency of PD-L1 immunohistochemistry as predictive test for immunotherapy in non-small cell lung cancer, J. Clin. Pathol. 73 (7) (2020) 423–430, https://doi.org/10.1136/jclinpath-2019-205993.
[44] Y.-L. Wu, D. Planchard, S. Lu, H. Sun, N. Yamamoto, D.-W. Kim, D.S.W. Tan, J.C.-H. Yang, M. Azrif, T. Mitsudomi, K. Park, R.A. Soo, J.W.C. Chang, A. Alip, S. Peters, J.-Y. Douillard, Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS, Ann. Oncol. 30 (2) (2019) 171–210, https://doi.org/10.1093/annonc/mdy554.
[45] National Cancer Institute. Non-Small Cell Lung Cancer Treatment (PDQ®)–Health Professional Version, 2021. Available from: https://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq#_48406. Accessed 7 June 2021.
[46] J. Adam, V. Hofman, A. Mansuet-Lupo, I. Rouquette, J. Vignaud, C. Badoual, H. Bégueret, M. Copin, D. Damotte, C. Danel, F. Forest, S. Garcia, L. Gibault, F. Penault-Llorca, S. Lantuejoul, A. Cazes, N. Le Stang, P2.09-17 real-world concordance across pathologists for PD-L1 scoring in non-small cell lung cancer: results from a large nationwide initiative, J. Thoracic Oncol. 14 (10) (2019) S775, https://doi.org/10.1016/j.jtho.2019.08.1666.
[47] V.H. Koelzer, A. Gisler, J.C. Hanhart, et al., Digital image analysis improves precision of PD-L1 scoring in cutaneous melanoma, Histopathology 73 (3) (2018) 397–406, https://doi.org/10.1111/his.2018.73.issue-310.1111/his.13528.
[48] C. Keppens, E.M. Dequeker, P. Pauwels, A. Ryska, N. 't Hart, J.H. von der Thüsen, PD-L1 immunohistochemistry in non-small-cell lung cancer: unraveling differences in staining concordance and interpretation, Virchows Arch. 478 (5) (2021) 827–839, https://doi.org/10.1007/s00428-020-02976-5.
[49] E. Thunnissen, How to validate predictive immunohistochemistry testing in pathology? A practical approach exploiting the heterogeneity of programmed death ligand-1 present in non-small cell lung cancer, Arch. Pathol. Lab. Med. 143 (1) (2019) 11–12, https://doi.org/10.5858/arpa.2018-0410-ED.
[50] D. Li, X. Zhu, H. Wang, N. Li, Association between PD-L1 expression and driven gene status in NSCLC: a meta-analysis, Eur. J. Surg. Oncol. 43 (7) (2017) 1372–1379, https://doi.org/10.1016/j.ejso.2017.02.008.
[51] F. Petrelli, M. Maltese, G. Tomasello, et al., Clinical and molecular predictors of PD-L1 expression in non-small-cell lung cancer: systematic review and meta-analysis, Clin. Lung Cancer 19 (4) (2018) 315–322, https://doi.org/10.1016/j.cllc.2018.02.006.
[52] M. Zhang, G. Li, Y. Wang, et al., PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis, Sci. Rep. 7 (1) (2017), https://doi.org/10.1038/s41598-017-10925-7.
[53] P. Song, L. Guo, W. Li, F. Zhang, J. Ying, S. Gao, Clinicopathologic correlation with expression of PD-L1 on both tumor cells and tumor-infiltrating immune cells in patients with non-small cell lung cancer, J. Immunother. 42 (1) (2019) 23–28, https://doi.org/10.1097/CJI.0000000000000249.
[54] B.G. Skov, S.B. Rorvig, T.H.L. Jensen, T. Skov, The prevalence of programmed death ligand-1 (PD-L1) expression in non-small cell lung cancer in an unselected, consecutive population, Mod. Pathol. 33 (1) (2020) 109–117, https://doi.org/10.1038/s41379-019-0339-0.
[55] L. Ye, C. Leslie, A. Jacques, N. Mesbah Ardakani, B. Amanuel, M. Millward, Programmed death ligand-1 expression in non-small cell lung cancer in a Western Australian population and correlation with clinicopathologic features, Mod. Pathol. 32 (4) (2019) 524–531, https://doi.org/10.1038/s41379-018-0173-9.