Conventional PCR-based versus next-generation sequencing-based approach for T-cell receptor γ gene clonality assessment in mature T-cell lymphomas: A phase 3 diagnostic accuracy study

SUPPLEMENTARY FILE

Supplementary Table 1. STARD requirements

Section and Topic	Item #		On page #
Title/Abstract/ Keywords	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading "sensitivity and specificity").	2,3
Introduction	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	2,3
Methods			
Participants	3	Describe the study population: The inclusion and exclusion criteria, setting and locations where the data were collected.	3
	4	Describe participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	3
	5	Describe participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected.	3
	6	Describe data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	3
Test methods	7	Describe the reference standard and its rationale.	5,6
	8	Describe technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	4-6
	9	Describe definition of and rationale for the units, cutoffs, and/or categories of the results of the index tests and the reference standard.	7
	10	Describe the number, training, and expertise of the persons executing and reading the index tests and the reference standard.	5,6
	11	Describe whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	5,6
Statistical methods	12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	7
	13	Describe methods for calculating test reproducibility, if done.	/
Results			
Participants	14	Report when study was done, including beginning and ending dates of recruitment.	2012–2014
	15	Report clinical and demographic characteristics of the study population (e.g., age, sex, spectrum of presenting symptoms, comorbidity, current treatments, and recruitment centers).	/
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	3, 7-9
Test results	17	Report time interval from the index tests to the reference standard, and any treatment administered between.	Samples, who received the reference standard test at diagnosis (2012-2014), were then analyzed with index test at the time of the study (2014).
	18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	7-9
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	4, 5, Table 1
	20	Report any adverse events from performing the index tests or the reference standard.	3
Estimates	21	Report estimates of diagnostic accuracy and measures of statistical uncertainty (e.g., 95% confidence intervals).	7-9, 11, Table 3
	22	Report how indeterminate results, missing responses and outliers of the index tests were handled.	11
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	11
	24	Report estimates of test reproducibility, if done.	/
Discussion	25	Discuss the clinical applicability of the study findings.	10-13

Supplementary Table 2. QUADAS 2 requirements

Phase 1: State the review question:	
Patients (setting, intended use of index test, presentation, and prior testing):	49 T-cell non-Hodgkin lymphomas (T-NHL) and 23 reactive lymphoid hyperplasia with paracortical expansion (RLH), collected from formalin-fixed and paraffin-embedded (FFPE) tissue (*n*=49) or fresh/frozen tissue (*n*=23) were included. The index tests were performed to assess the diagnostic accuracy of NGS technologies (Invivoscribe LymphoTrack® Dx TRG MiSeq® Assay) to evaluate clonality.
Index test (s):	Invivoscribe LymphoTrack® Dx TRG MiSeq® Assay (NGS)
Reference standard and target condition:	Reference standard was performed by polymerase chain reaction (PCR) assays followed by heteroduplex analysis/capillary electrophoresis and/or Sanger sequencing according to the BIOMED-2 guidelines.
Phase 2: Draw a flow diagram for the primary study	
Domain 1: Patient Selection	
A. Risk of Bias	
Describe methods of patient selection:	
Was a consecutive or random sample of patients enrolled?	Non-consecutive
Was a case–control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Yes
Could the selection of patients have introduced bias?	Yes, samples originated from suspicious patients.
B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test, and setting):	72 samples of T-lymphoproliferative disorder were analyzed by index test and reference standard to assess the diagnostic accuracy of NGS technologies in clonality evaluation of TRG. Diagnoses (made with the reference standard) were reviewed by at least two qualified molecular hematopathologists. The index test was applied retrospectively to all the cases.
Is there concern that the included patients do not match the review question?	Concern: low
Domain 2: Index Test (S) TRG Clonality Analysis by NGS versus PCR	
A. Risk of Bias	
Describe the index test and how it was conducted and interpreted:	We compared the index test to reference standard to verify its diagnostic accuracy concerning clonality assessment of TRG. The results were interpreted according to BIOMED-2 and Invivoscribe guidelines.
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes
If a threshold was used, was it pre-specified?	Yes
Could the conduct or interpretation of the index test have introduced bias?	Risk: low
B. Concerns regarding applicability	
Is there concern that the index test, its conduct, or interpretation differ from the review question?	Concern: low
Domain 3: Reference Standard	
A. Risk of Bias	
Describe the index test and how it was conducted and interpreted:	Polymerase chain reaction (PCR) assays, followed by capillary electrophoresis and/or Sanger sequencing were performed and interpreted according to BIOMED-2 guidelines. These tests use a mixture of consensus primers designed to amplify and to sequence the majority of possible unique V-J rearrangements of TRG locus.
Is the reference standard likely to correctly classify the target condition?	Yes, with exceptions
Were the reference standard results interpreted without knowledge of the results of the index test?	Yes
Could the reference standard, its conduct, or its interpretation have introduced bias?	Risk: low
B. Concerns regarding applicability	
Is there concern that the target condition as defined by the reference standard does not match the review question?	Concern: low

(*Cont'd...*)

Supplementary Table 2. (*Continued*)

Domain 4: Flow And Timing	
A. Risk of Bias	
Describe any patients who did not receive the index test (s) and/or reference standard or who were excluded from the 2×2 table (refer to flow diagram)	97 out of 169 collected samples, unfortunately, were not evaluable due to an insufficient amount of DNA, an excessive degradation and/or impurity or insufficient NGS reads (<10,000).
Describe the time interval and any interventions between index test (s) and reference standard:	Samples were collected at diagnosis, when the reference standard was performed. At the time of the study (2014), index test was performed on the same cases.
Was there an appropriate interval between index test (s) and reference standard?	Yes
Did all patients receive a reference standard?	Yes
Did patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No (97 out of 169)
Could the patient flow have introduced bias?	Risk: low

Supplementary Table 3. REMARK requirements

Guidelines for the REporting of tumor MARKer Studies (REMARK)	See page
Introduction	
1. State the marker examined, the study objectives, and any prespecified hypotheses.	2, 3
Materials and Methods	
Patients	
2. Describe the characteristics (e.g., disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria.	3
3. Describe treatments received and how chosen (e.g., randomized or rule-based).	Not applicable
Specimen characteristics	
4. Describe the type of biological material used (including control samples) and methods of preservation and storage.	3
Assay methods	
5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study end point.	3-7
Study design	
6. State the method of case selection, including whether the study design was prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	3, 2012-2014
7. Precisely define all clinical end points examined.	Accuracy, ST, SP, PPV, NPV, LR+, LR-
8. List all candidate variables initially examined or considered for inclusion in models.	7
9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	Sample size representative for routine diagnostic cases
Statistical analysis methods	
10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	7
11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cut point determination.	7
Results	
Data	
12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	3, 7, 8
13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	Not applicable
Analysis and presentation	
14. Show the relation of the marker to standard prognostic variables.	Not applicable
15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan–Meier plot is recommended.	Table 1

(*Cont'd...*)

Supplementary Table 3. (*Continued*)

Guidelines for the REporting of tumor MARKer Studies (REMARK)	See page
16. For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	7-9
17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	Table 2 and 3
18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	/
Discussion	
19. Interpret the results in the context of the prespecified hypotheses and other relevant studies; include a discussion of limitations of the study.	10-12
20. Discuss implications for future research and clinical value.	12, 13