Sequencing-based counting and size profiling of plasma Epstein-Barr virus DNA enhance population screening of Nasopharyngeal Carcinoma

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A blood test using a combination of qPCR and next-generation sequencing (NGS) detected Nasopharyngeal Carcinoma (NPC) using a single blood draw with comparable detection rates (sensitivity) and fewer false positives (specificity) compared with the original qPCR/two-blood draw assay developed in a large prospective cohort study that was described in the *New England Journal of Medicine* (*NEJM*)[1].

Clinical Summary

Background

A previous study, published in the *NEJM* in August 2017, showed that detection of Epstein-Barr virus (EBV) DNA in plasma has high sensitivity and specificity for NPC and that testing high risk persons results in identification of NPC at earlier stages of disease compared to historical rates[1] .

In the *NEJM* study, qPCR was used to test blood plasma samples for EBV DNA. If the test was positive, the person was asked to return four weeks later for a second blood draw and another qPCR EBV DNA test. Individuals with positive results across the two tests were suspected of having NPC and referred for evaluation with nasal endoscopy and/or MRI. 11% of those were confirmed as having NPC. This early detection strategy identified 34/35 (97.1%) of NPC cases with a specificity of 98.6%. 71% of people diagnosed with NPC in the study were diagnosed at stage 1 or 2 compared with 20% from historical data ($p < 0.001$).

Following the *NEJM* study, it was hypothesized that plasma EBV DNA may have molecular characteristics that could differentiate between individuals who actually have NPC and those who had false-positive results (did not have NPC but had positive test results with the conventional qPCR assay) thereby enhancing test performance. Moreover, if these molecular differences could be detected in the first blood draw, this would reduce the logistical issues and potential for non-compliance with the clinical protocol introduced by getting blood draws at two separate time points.

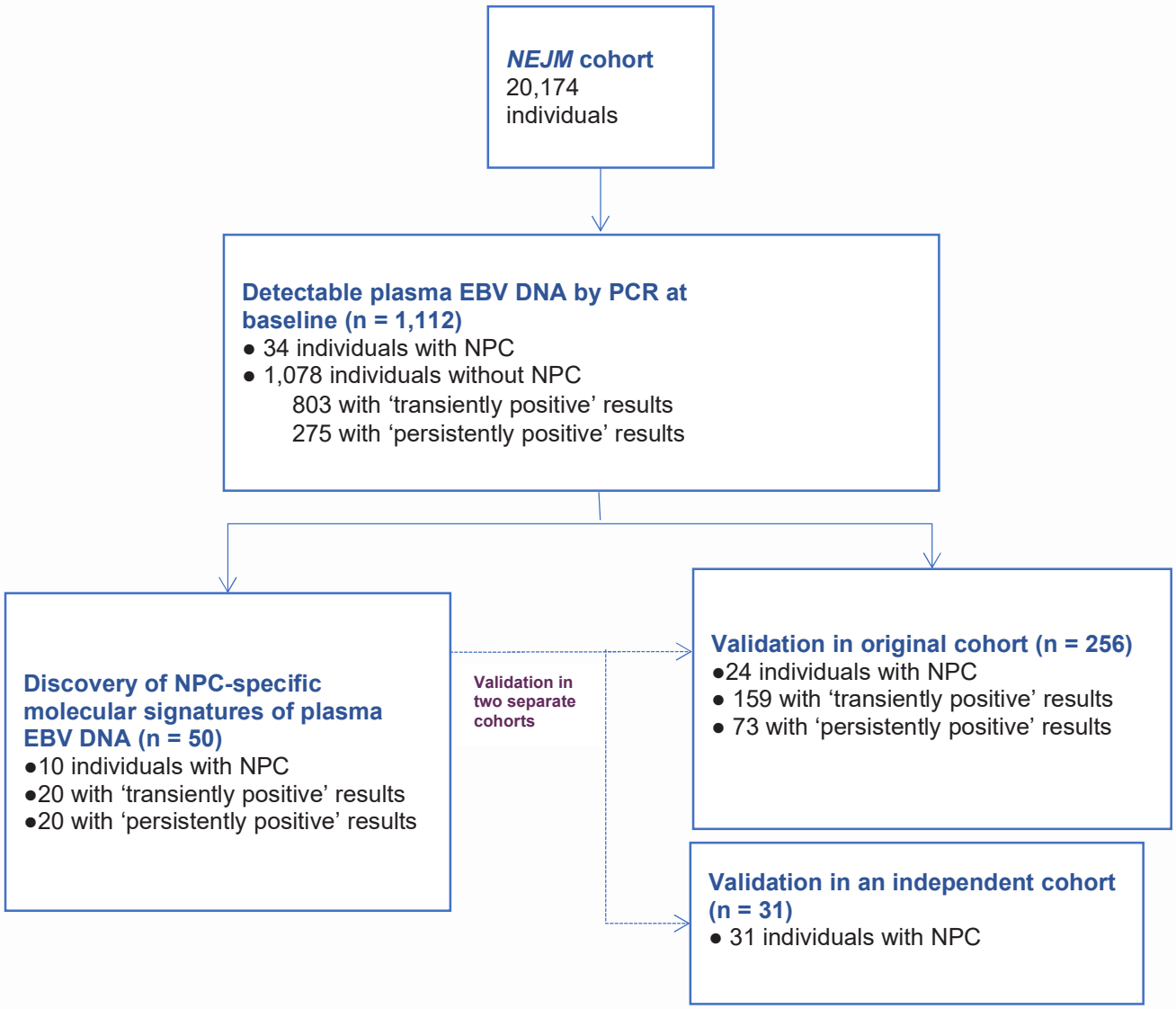
Objectives of Current Study

- Identify plasma EBV DNA molecular characteristics that would maintain sensitivity while enhancing specificity by better differentiating persons with NPC from persons who do not have NPC but are positive for plasma EBV DNA.
- Demonstrate that blood-based detection of Nasopharyngeal cancer can be performed using a single time-point plasma EBV DNA test with high accuracy for early stage disease.

Study Design

● Plasma samples of people with and without NPC were selected from the subset of 1,112 people who had detectable EBV DNA by qPCR at baseline in the NEJM study (see Figure 1).
– An exploratory sample set included 50 samples from the qPCR-positive subset, from 20 people with ‘transiently positive’ results who did not have NPC, 20 people with ‘persistently positive’ results who did not have NPC, and 10 people with NPC (5 stage I, 2 stage II, 2 stage III, 1 stage IV).
– The validation sample set included 256 samples from the qPCR-positive subset (from randomly selected people with ‘transiently positive’ results who did not have NPC (159), randomly selected people with ‘persistently positive’ results who did not have NPC (73), and 24 people with NPC (11 stage I, 6 stage II, 6 stage III, 1 stage IV).
● An additional 31 individuals with NPC (3 stage I, 2 stage II, 20 stage III, 6 stage IV) from an independent cohort were also tested in the validation set. These patients had presented symptomatically for NPC and were recruited for the study.

Figure 1: Study cohorts



Results (see figure 2)

● Diagnostic performance of a new protocol, incorporating this combined analysis of plasma EBV DNA using NGS after qPCR, was modeled using the entire 20,174-person cohort of the *NEJM* study. Participants were defined as 'test-positive' if their plasma samples exceeded the cutoffs in both the count and size-based analyses. Otherwise, they were defined as 'test-negative.'

– Projected sensitivity of the new protocol was 97.1% (95% CI: 85.1%, 99.9%).

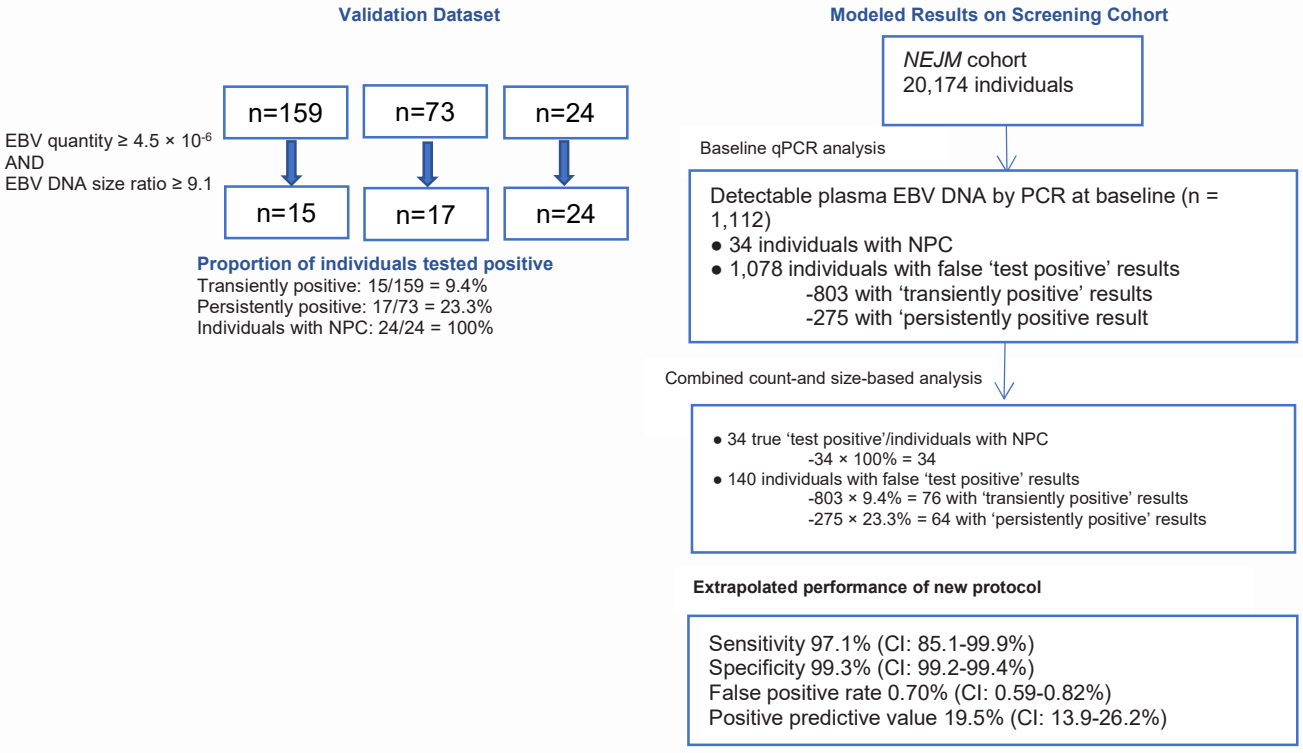
■ All 34 NPC cases that tested positive in the original study could be captured.

– Projected specificity was 99.3% (95% CI: 99.2%, 99.4%).

■ Only 140 false positives resulted from this method vs. 275 in the two-step qPCR EBV DNA approach; this method correctly identified 20,034/20,174 individuals as true negatives.

– PPV and false positive rate were estimated to be 19.5% (95% CI: 13.9%, 26.2%) and 0.70% (95% CI, 0.59%, 0.82%)

Figure 2: Modeling the performance of sequencing-based analysis of plasma EBV DNA in the entire 20,174-person cohort



Conclusions

● Differentiating molecular characteristics of plasma EBV DNA between people with and without NPC can be used for accurate detection of NPC via a single time-point blood draw.

● Using NGS to identify NPC-associated EBV DNA patterns enables a blood-based early detection test that is more accurate (has the same sensitivity but reduces false positives) than existing alternatives while only requiring a single blood draw.

● This approach reduces the likelihood of unnecessary diagnostic workup, and reduces the logistical and potential compliance issues introduced by running two tests at two separate time points compared to the conventional qPCR assay.

[1] Chan KCA, Woo JKS, King A, et al. Analysis of plasma Epstein–Barr virus DNA to screen for nasopharyngeal cancer. *N Engl J Med*. 2017;377(6):513-522.

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