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Analysis of Plasma Epstein–Barr Virus DNA to Screen for Nasopharyngeal Cancer

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ABSTRACT

BACKGROUND

Circulating cell-free Epstein–Barr virus (EBV) DNA is a biomarker for nasopharyngeal carcinoma. We conducted a prospective study to investigate whether EBV DNA in plasma samples would be useful to screen for early nasopharyngeal carcinoma in asymptomatic persons.

METHODS

We analyzed EBV DNA in plasma specimens to screen participants who did not have symptoms of nasopharyngeal carcinoma. Participants with initially positive results were retested approximately 4 weeks later, and those with persistently positive EBV DNA in plasma underwent nasal endoscopic examination and magnetic resonance imaging (MRI).

RESULTS

A total of 20,174 participants underwent screening. EBV DNA was detectable in plasma samples obtained from 1112 participants (5.5%), and 309 (1.5% of all participants and 27.8% of those who initially tested positive) had persistently positive results on the repeated sample. Among these 309 participants, 300 underwent endoscopic examination, and 275 underwent both endoscopic examination and MRI; of these participants, 34 had nasopharyngeal carcinoma. A significantly higher proportion of participants with nasopharyngeal carcinoma that was identified by screening had stage I or II disease than in a historical cohort (71% vs. 20%, P<0.001 by the chi-square test) and had superior 3-year progression-free survival (97% vs. 70%; hazard ratio, 0.10; 95% confidence interval, 0.05 to 0.18). Nine participants declined to undergo further testing, and 1 of them presented with advanced nasopharyngeal carcinoma 32 months after enrollment. Nasopharyngeal carcinoma developed in only 1 participant with negative EBV DNA in plasma samples within 1 year after testing. The sensitivity and specificity of EBV DNA in plasma samples in screening for nasopharyngeal carcinoma were 97.1% and 98.6%, respectively.

CONCLUSIONS

Analysis of EBV DNA in plasma samples was useful in screening for early asymptomatic nasopharyngeal carcinoma. Nasopharyngeal carcinoma was detected significantly earlier and outcomes were better in participants who were identified by screening than in those in a historical cohort. (Funded by the Kadoorie Charitable Foundation and the Research Grants Council of the Hong Kong government; ClinicalTrials.gov number, NCT02063399.)

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Analysis of circulating DNA derived from cancer cells, which is frequently known as a "liquid biopsy," is being evaluated as a tool in the care of patients with cancer.[1] Although a wide range of cancer-associated changes, including point mutations,[2-5] copy-number aberrations,[2,6,7] and alterations in DNA methylation,[6,8,9] have been detected in the plasma of patients with cancer, the clinical applications of analysis of circulating tumor DNA have thus far been focused primarily on guiding treatment selection and detection of residual disease.[4,10,11] For example, the detection of mutations in the gene encoding the epidermal growth factor receptor (*EGFR*) in plasma has been used to guide treatment with EGFR tyrosine kinase inhibitors.[12,13] Patient-specific alterations have been used to identify minimal residual disease[4] and monitor disease progression after treatment.[4,11]

There is much less information on the use of analysis of circulating DNA to screen for early cancers. A fundamental question is whether a small tumor would release sufficient amounts of tumor DNA into the circulation to allow sensitive detection of the cancer-associated changes. Most existing tumor markers that are used for cancer screening are proteins or glycoproteins (e.g., prostate-specific antigen for prostate cancer and alpha-fetoprotein for hepatocellular carcinoma).

In this study, we used nasopharyngeal carcinoma as a model to show the potential application of analysis of circulating DNA for cancer screening. Nasopharyngeal carcinoma is prevalent in Southeast Asia.[14] Among middle-aged men, the incidence of nasopharyngeal carcinoma in endemic areas is up to 35 cases per 100,000 persons,[15] and risk factors include a family history of nasopharyngeal carcinoma, consumption of salted fish, and smoking.[16] The pathogenesis of nasopharyngeal carcinoma is closely associated with Epstein–Barr virus (EBV), and circulating cancer-derived EBV DNA in plasma has been established as a tumor marker for nasopharyngeal carcinoma, with a sensitivity of 96% and a specificity of 93%.[17,18] EBV DNA in plasma consists of short DNA fragments (primarily <181 bp) that are released by nasopharyngeal carcinoma cells, rather than being associated with viral particles.[19]

The extent of nasopharyngeal carcinoma at diagnosis is the most important factor affecting survival. The 5-year survival among patients with stage I disease is as high as 95%, whereas survival among patients with stage IV disease is just over 60%.[20,21] Unfortunately, since early nasopharyngeal carcinoma is relatively asymptomatic, 80% of the patients with nasopharyngeal carcinoma present with locally advanced disease or distant metastasis at diagnosis.[21,22]

The identification of patients with early-stage nasopharyngeal carcinoma through screening could potentially improve treatment outcomes. Although anti-EBV IgA serologic tests are commonly performed to evaluate patients in whom nasopharyngeal carcinoma is suspected, there is little information on the use of these markers to screen for nasopharyngeal carcinoma in asymptomatic participants, possibly because of the low sensitivities and specificities of such markers.[23,24] Proof-of-principle studies have shown that analysis of EBV DNA in plasma could potentially detect small asymptomatic nasopharyngeal carcinomas.[25,26]

Here, we present a prospective study involving more than 20,000 participants in whom analysis of EBV DNA in plasma was performed to screen for nasopharyngeal carcinoma. We aimed to address whether this analysis could detect nasopharyngeal carcinoma among asymptomatic persons and whether the identified cases of disease would be in an early stage.

METHODS

PARTICIPANTS

We organized public health education sessions in Hong Kong between July 2013 and February 2016. These sessions provided information regarding the epidemiologic features and treatment of nasopharyngeal carcinoma. After each session, ethnically Chinese men who were 40 to 62 years of age were invited to participate in the study, since the incidence of nasopharyngeal carcinoma is highest in this group and decreases among persons in older age groups. Participants with a history of cancer or autoimmune conditions and those receiving systemic glucocorticoid or immunosuppressive therapy were excluded in order to minimize the chance of having false positive results in plasma EBV DNA associated with EBV viral replication in immunocompromised participants.[19,25]

STUDY DESIGN AND SAMPLE PROCESSING

A sample containing 20 ml of venous blood was obtained from each participant at enrollment. EBV DNA in plasma was analyzed by means of real-time polymerase-chain-reaction (PCR) assay that targeted the *Bam*HI-W fragment of the EBV genome, as described previously.[17,27] The lower detection limit of the assay was 20 EBV genomes per milliliter of plasma.[17,27] All the samples were analyzed in duplicate. Amplification signals in any replicate were regarded as a positive result, regardless of the level. In participants with positive results, another blood sample was obtained approximately 4 weeks later.

Participants with persistently positive results, defined as "screen-positive," were referred for endoscopic examination and magnetic resonance imaging (MRI) of the nasopharynx.[28] This two-stage testing arrangement aimed to differentiate cases of nasopharyngeal carcinoma from false positive cases, since participants with nasopharyngeal carcinoma have persistently positive results, whereas participants without nasopharyngeal carcinoma tend to have transiently positive results.[25] To test for the reproducibility of the assay, 20 plasma samples with positive EBV DNA and 20 with negative results were randomly selected for reanalysis. All the samples showed results that were the same as those of the initial tests.

All endoscopic examinations were performed by one otorhinolaryngologist. Biopsy specimens were obtained from all suspicious lesions. All MRI scans were reviewed by a radiologist with specialized training in MRI of the head and neck. The clinical stages of the participants were determined on the basis of MRI findings according to the tumor–node–metastasis staging system of the International Union Against Cancer and the American Joint Committee on Cancer (AJCC), as described in the seventh edition of the AJCC cancer-staging manual.[29] After enrolling in the study, all the participants were interviewed by telephone annually.

STUDY OVERSIGHT

The study protocol, available with the full text of this article at NEJM.org, was approved by the joint ethics committee of the Chinese University of Hong Kong–New Territories East Cluster. All the participants provided written informed consent. A scientific advisory board with international advisors was formed to monitor the study. The first and last authors designed the study, wrote the first draft of the manuscript, and made the decision to submit the manuscript for publication. All the authors collected and analyzed the data, contributed to the drafting of the manuscript, and vouch for the completeness and accuracy of the data, the accuracy of the analyses, and the fidelity of the study to the protocol. No one who is not an author contributed to the manuscript.

STATISTICAL ANALYSIS

The Kaplan–Meier method was used to compare survival among the participants with nasopharyngeal carcinoma identified by screening with survival among those identified in a historical cohort.[20] The survival data from the historical cohort were derived from a previous study involving 2687 consecutive patients who received treatment at all public oncology centers in Hong Kong over a period of 5 years.[20] A subgroup of 1278 men who were 40 to 62 years of age was used for comparison. The survival and stage distribution among patients in the historical cohort were similar to the results reported recently by another center.[21]

RESULTS

PARTICIPANTS

A total of 20,349 participants were enrolled from 147 health education sessions, and 175 participants were excluded from further assessment (Fig. 1). A total of 120 of the participants attempted to enroll twice and were excluded. For these participants, only the first plasma sample was processed for further assessment. Nine participants were excluded because they had concurrent cancer, and 46 were excluded because they had autoimmune diseases or were receiving glucocorticoids or immunosuppressive therapy.

SCREEN-POSITIVE PARTICIPANTS

The demographic characteristics of the 20,174 eligible participants are listed in Table 1. At enrollment, 1112 participants (5.5%) had detectable EBV DNA in plasma and received follow-up analysis of EBV DNA in plasma. The median interval between the first and the follow-up tests

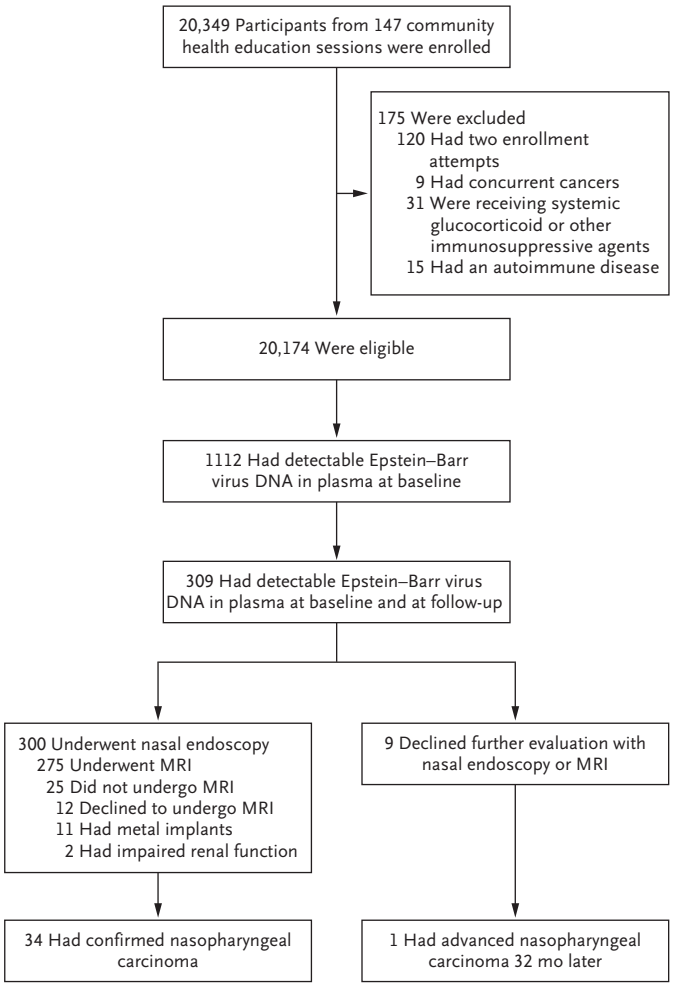

Figure 1. Enrollment, Screening of Epstein–Barr Virus DNA in Plasma, and Confirmation of Nasopharyngeal Carcinoma by Means of Nasal Endoscopy and Magnetic Resonance Imaging in the Participants.

A total of 20,349 participants were recruited into the study. Analysis of EBV DNA in plasma was used to screen for nasopharyngeal carcinoma in eligible participants, and 34 cases of nasopharyngeal carcinoma were detected.

was 34 days (interquartile range, 30 to 40 days). Among the 309 participants who had persistently positive results, 300 underwent endoscopic examination and 275 also underwent MRI (Fig. 1). Twelve participants declined to undergo MRI but underwent endoscopic examination. Nine participants declined to undergo any further evaluation. Thirteen participants had contraindications to MRI (Fig. 1).

Histologically proven undifferentiated nasopharyngeal carcinoma was confirmed in 34 (11%) of the 300 participants who underwent further assessment. Figure 2A shows the stage distribution in these participants as compared with all patients with nasopharyngeal carcinoma in Hong Kong as recorded in the 2013 Hong Kong Cancer Registry.[22] A significantly higher proportion of these participants had stage I and II disease than those in a historical cohort (71% vs. 20%, P<0.001 by the chi-square test). The median follow-up in these 34 participants was 24 months. Disease recurrence after treatment developed in only 1 participant. The 3-year rate of progression-free survival among the participants with nasopharyngeal carcinoma that was detected on screening was superior to that among the patients in the historical cohort (97% vs. 70%; hazard ratio, 0.10; 95% confidence interval, 0.05 to 0.18; P<0.001 by the log-rank test) (Fig. 2B).[20]

In three participants, the tumor was not detected on the initial endoscopic examination but was identified on MRI. As shown in the endoscopic examination (Fig. 3A) and the MRI scan (Fig. 3B) of one patient with a tumor located at the right pharyngeal recess, the epithelial lining was smooth and there was no obvious mass or outgrowth. Because of the abnormal MRI findings, a second nasal endoscopy was performed in each patient and a biopsy specimen was obtained from the region where the abnormality was detected.

Among the 266 participants with positive EBV DNA in plasma but no nasopharyngeal carcinoma identified by means of endoscopy and MRI, 195 had normal endoscopic findings and 71 had lymphoid tissues (a common observation even in healthy participants). Biopsies were performed in 32 participants with prominent lymphoid tissues, and all were negative for cancer.

FOLLOW-UP

All enrolled participants were interviewed by telephone annually to determine whether nasopharyngeal carcinoma had developed. The median duration of follow-up was 22 months (range, 12 to 44 months). As of March 31, 2017, the total follow-up of the cohort was 40,909 person-years.

A 55-year-old participant in whom EBV DNA was detected in plasma at baseline and at 4 weeks but who declined to undergo nasal endoscopy and MRI received a diagnosis of advanced-stage nasopharyngeal carcinoma with a presentation of multiple enlarging neck lumps at 32 months after enrollment; this participant died 2 months

Table 1. Demographic Characteristics of the Study Participants.*

Characteristic	All Eligible Participants (N=20,174)	Plasma EBV DNA–Positive at Baseline (N=1112)	Plasma EBV DNA–Positive at Baseline and at Follow-up (N=309)	Confirmed Nasopharyngeal Carcinoma (N=34)	Historical Cohort (N=1278)†
Age — yr					
Median	52	53	53	51	48
Interquartile range	46–56	48–58	47–57	44–55	44–55
Age distribution — no. (%)					
40–44 yr	3,901 (19.3)	172 (15.5)	50 (16.2)	9 (26.5)	392 (30.7)
45–49 yr	3,972 (19.7)	198 (17.8)	60 (19.4)	5 (14.7)	312 (24.4)
50–54 yr	5,295 (26.2)	268 (24.1)	71 (23.0)	10 (29.4)	240 (18.8)
55–59 yr	4,680 (23.2)	288 (25.9)	79 (25.6)	9 (26.5)	221 (17.3)
60–62 yr	2,326 (11.5)	186 (16.7)	49 (15.9)	1 (2.9)	113 (8.8)
Smoking status — no. (%)					
Never smoked	12,665 (62.8)	634 (57.0)	165 (53.4)	25 (73.5)	
Current or recent smoker‡	4,045 (20.1)	296 (26.6)	93 (30.1)	8 (23.5)	
Stopped smoking for >1 yr	3,463 (17.2)	182 (16.4)	51 (16.5)	1 (2.9)	
Drinking status — no. (%)					
Nondrinker	7,071 (35.1)	381 (34.3)	116 (37.5)	17 (50.0)	
Drinker	13,103 (64.9)	731 (65.7)	193 (62.5)	17 (50.0)	
Units of alcohol consumed/wk — no. (%)					
<1	7,594 (37.6)	427 (38.4)	106 (34.3)	8 (23.5)	
1–5	4,055 (20.1)	213 (19.2)	63 (20.4)	6 (17.6)	
6–10	892 (4.4)	55 (4.9)	13 (4.2)	2 (5.9)	
>10	562 (2.8)	36 (3.2)	11 (3.6)	1 (2.9)	
History of nasopharyngeal carcinoma in first-degree relative — no. (%)					
No	19,171 (95.0)	1010 (90.8)	280 (90.6)	26 (76.5)	
Yes	1,003 (5.0)	102 (9.2)	29 (9.4)	8 (23.5)	
In parents	759 (3.8)	66 (5.9)	18 (5.8)	4 (11.8)	
In siblings	250 (1.2)	40 (3.6)	11 (3.6)	4 (11.8)	
In children	2 (<0.1)	0	0	0	

* EBV denotes Epstein–Barr virus.
† Data regarding smoking and drinking status and family history of nasopharyngeal carcinoma were not available for the historical cohort.
‡ The category of current or recent smoker includes those who stopped smoking within 1 year before enrollment.

later. Two participants who had undetectable EBV DNA in plasma at baseline reported at annual interviews that nasopharyngeal carcinoma had developed. The first patient had a 20-year history of allergic rhinitis. Because of persistent rhinorrhea, he consulted an otorhinolaryngologist at 4 months after enrollment, and stage II nasopharyngeal carcinoma was diagnosed. The second participant noticed an enlarging neck lump at 22 months after enrollment and received a diagnosis of stage III nasopharyngeal carcinoma. Since nasopharyngeal carcinoma developed in only 1 of the 19,865 screen-negative persons within 1 year after screening, the sensitivity and negative predictive value for this screening strategy were 97.1% and 99.995%, respectively. The diagnostic performance of the screening program is summarized in Table 2.

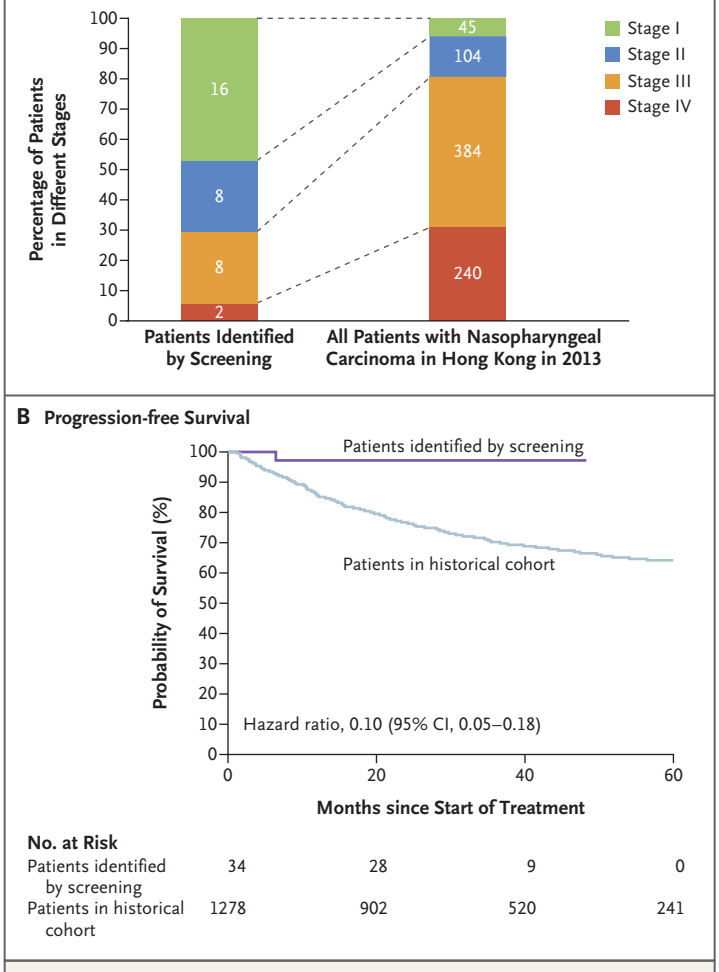

Figure 2. Stage Distribution and Progression-free Survival among the Participants with Nasopharyngeal Carcinoma Identified by Screening.

Panel A shows the distribution of disease stage among the 34 participants with nasopharyngeal carcinoma. The stage distribution in all the patients with nasopharyngeal carcinoma in Hong Kong in 2013 according to the Hong Kong Cancer Registry is shown as a comparison. The number of cases for each stage is shown. Panel B shows the rate of progression-free survival among the participants as compared with 1278 age- and sex-matched patients in a historical cohort in Hong Kong, which is a subgroup of the study cohort described by Lee et al.[20] CI denotes confidence interval.

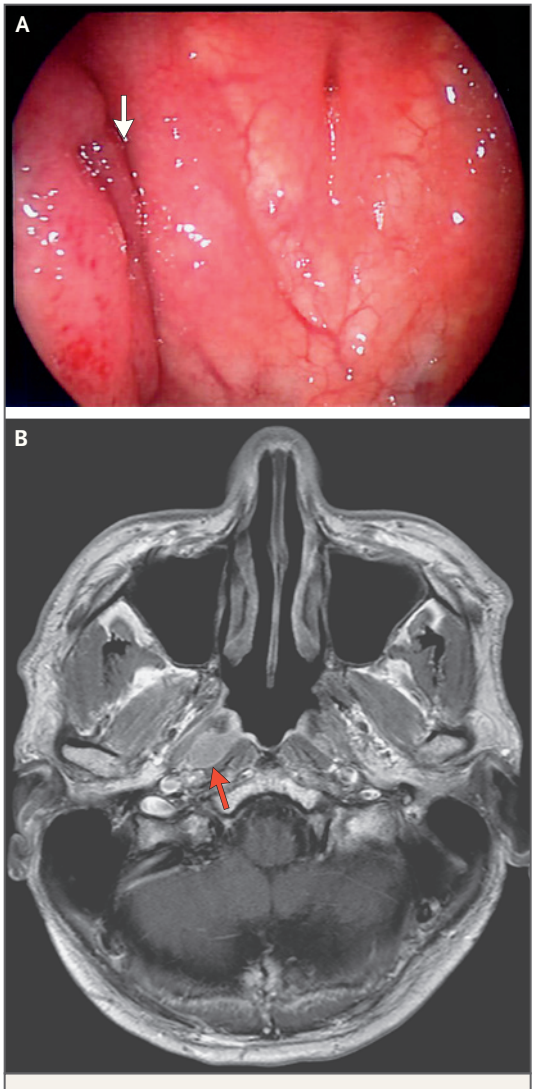

Figure 3. Findings on Nasal Endoscopy and Axial T_1-Weighted MRI of a 48-Year-Old Man with a Small Nasopharyngeal Carcinoma in the Right Pharyngeal Recess (Stage T1).

As shown in Panel A, no tumor was observed on the endoscopic examination. The epithelial lining was smooth, and the right pharyngeal recess was preserved (white arrow). As shown in Panel B, a small tumor in the right pharyngeal recess (red arrow) was detected on MRI performed after the administration of contrast material.

DISCUSSION

Using nasopharyngeal carcinoma as a model, we found that it was feasible to use analysis of circulating DNA to screen for cancers in asymptomatic persons. Of the 20,174 participants who underwent screening, only 309 (1.5% of all participants and 27.8% of those who initially tested positive) had persistently detectable EBV DNA in plasma at baseline and at follow-up. Among these 309 participants, nasopharyngeal carcinoma was confirmed in 34 (11.0%). Low positive predictive values are typical for cancer screening studies performed in asymptomatic populations. For example, a recent Korean study that screened 45,855 asymptomatic participants

Table 2. Sensitivity and Specificity of the Two-Stage Screening Protocol for the Detection of Nasopharyngeal Carcinoma.*

Finding	Screen-Positive (N=308)†	Screen-Negative (N=19,865)
Confirmed nasopharyngeal carcinoma by the screening protocol or nasopharyngeal carcinoma reported to have developed within 1 yr — no.	34	1
No nasopharyngeal carcinoma within 1 yr after screening — no.	274	19,864
Sensitivity — % (95% CI)	97.1 (95.5–98.7)	
Specificity — % (95% CI)	98.6 (98.6–98.7)	
Positive predictive value — % (95% CI)	11.0 (10.7–11.3)	
Negative predictive value — % (95% CI)	99.995 (99.99–100.00)	
Proportion of stage I/II disease in the 34 cases of nasopharyngeal carcinoma identified by screening — % (95% CI)	70.6 (69.6–72.5)	

* Screen-positive is defined as persistently positive for plasma EBV DNA at baseline and at follow-up. Screen-negative is defined as negative for plasma EBV DNA either at baseline or at follow-up.
† The participant who had declined further investigation but in whom advanced nasopharyngeal carcinoma developed 32 months after screening is not included in this number.

for hepatocellular carcinoma with the use of alpha-fetoprotein analysis showed a positive predictive value of only 1.66%.[30] The positive predictive value of 11% in this study is superior to the typical 3% value of existing blood-based tumor markers in a population-screening context.[31] Furthermore, nasal endoscopic examination is safe, quick, and inexpensive as compared with tests to confirm most other solid tumors.

The screening for nasopharyngeal carcinoma identified participants with early-stage disease. Remarkably, 16 participants (47%) had stage I disease. This proportion is substantially higher than the typical proportion of 5 to 7% in historical cohorts.[21,22] Between 2006 and 2010, of 2671 consecutive participants with nasopharyngeal carcinoma who received treatment at the Sun Yat-sen University Cancer Center, the largest treatment center for nasopharyngeal carcinoma in China, only 120 participants (4.5%) had stage I disease.[21] In patients with stage I disease, lesions that are localized in the nasopharynx can be effectively treated by means of intensity-modulated radiotherapy alone so that the side effects associated with more extensive radiotherapy and chemotherapy can be avoided.[32]

The 3-year rate of progression-free survival among the participants in whom nasopharyngeal carcinoma was identified by screening was superior to that among those in the historical cohort (97% vs. 70%; hazard ratio, 0.10).[20] In this study, the effect of length-time bias (which can occur when the lengths of intervals are analyzed by means of random selection or with respect to relatively nonprogressive cancers) is likely to be small because nasopharyngeal carcinoma is an aggressive cancer with frequent early progression and metastases.[33,34] Even if a patient presents with carcinoma in situ at the time of diagnosis, the disease often progresses to invasive nasopharyngeal carcinoma in 40 to 48 months. The potential confounding effect of lead-time bias (in this case, the interval between early diagnosis with screening and later diagnosis with standard techniques) could be addressed only in randomized, controlled trials. However, because curative treatment for early-stage nasopharyngeal carcinoma is available, the increase in progression-free survival is unlikely to be driven by an earlier diagnosis only, but more likely by the timely administration of effective treatments.

In this cohort of 20,174 participants, on the basis of an annual incidence of 35 cases per 100,000 persons in the target age group, nasopharyngeal carcinoma would be expected to develop in approximately 7 participants in a year.[22] The 34 cases identified is approximately the number of cases expected to be encountered in 5 years. One possible explanation is that participants who would originally present with more advanced nasopharyngeal carcinoma over the next few years had been identified by our screening program at earlier stages. This hypothesis is supported by the lower-than-expected number of

cases diagnosed during the follow-up period and the presentation of advanced-stage nasopharyngeal carcinoma in 1 screen-positive participant who had declined to undergo further assessment.

Among the 20,140 participants in whom nasopharyngeal carcinoma was not identified by screening, 19,626 (97.4%) were interviewed by telephone 1 year after screening, and nasopharyngeal carcinoma was reported to have developed in only 1 participant within the first year. This number is much lower than expected from the annual incidence. One of the 9 participants who declined further workup presented with an advanced nasopharyngeal carcinoma 32 months after enrollment. It is likely that the cancer had already been present in this participant at the time of screening. Had he not declined further assessment, the tumor might have been diagnosed much earlier and a better treatment outcome might have been expected.

In this study, screen-positive participants were assessed with the use of both nasal endoscopy and MRI. This arrangement can maximize the power for detecting nasopharyngeal carcinoma so as to provide the best ascertainment of the performance of screening to detect EBV DNA in plasma. In 3 participants, the tumors were not revealed on the endoscopic examination but were detected on MRI. These results are compatible with those in previous studies showing that MRI is more sensitive than nasal endoscopy for detecting small nasopharyngeal carcinomas in symptomatic patients.[28] If MRI screening is not readily available, assessment of test-positive participants with nasal endoscopy alone is a reasonable alternative, since this test could detect 91% (31 of 34) of the cancer cases.

The costs for each EBV DNA analysis, endoscopic examination, and MRI were $30, $80, and $1,000 (U.S. dollars), respectively. On the basis of the results of this study, to detect 1 case, 593 participants would need to be screened at a cost of $28,600. Considering the potential decrease in mortality and morbidity, as well as treatment-cost savings associated with the shift in stage distribution, screening for nasopharyngeal carcinoma appears to be a feasible practice in regions with a high incidence of this disease.

Although the current study focused only on men between the ages of 40 and 62 years, this screening protocol should also be applicable to women and persons in other age groups, in whom EBV DNA in plasma is also detected.[18,25] However, a lower positive predictive value would be expected owing to the lower incidence of nasopharyngeal carcinoma among women and persons in other age groups.

With a median follow-up of 22 months, the current study shows that the chance of the development of a nasopharyngeal carcinoma within 2 years after a negative screening is low. However, the most appropriate time interval for the screening can be addressed by a longer-term follow-up and rescreening of the cohort.

This study has shown the potential of analysis of circulating DNA to screen for early nasopharyngeal cancer. Even small tumors could release sufficient amounts of DNA into the circulation to allow sensitive detection. Since the half-life of EBV DNA clearance in plasma is only 2 hours,[35] to maintain an equilibrium level of EBV DNA in plasma at 20 EBV genomes per milliliter, the lower detection limit of our assay, 1 million EBV genomes need to be released from the tumor cells into the plasma each day. On the basis of the assumption that each tumor cell carries 50 genomes of EBV,[36] this is equivalent to a turnover of 200,000 cancer cells per day. This turnover of nasopharyngeal carcinoma cells is remarkable, given that 47% of the participants with disease that was identified through screening had stage I disease. Screening for cancers that are not associated with viral infections would be more challenging technically because detection of genetic and epigenetic markers other than viral DNA sequences would be needed. In each of the 50 genomes of EBV in nasopharyngeal carcinoma cells, there are approximately 10 repeats of the *Bam*HI-W region (our PCR target). Thus, we estimate that 500 molecular markers would be required to achieve a similar level of performance in detecting cancer-derived DNA as shown in this study.

In conclusion, we found that analysis of EBV DNA in plasma was useful for screening an at-risk population for nasopharyngeal carcinoma. The men with nasopharyngeal carcinoma who were identified by screening had significantly earlier stage distribution and superior progression-free survival than an unscreened population in a historical cohort.

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Drs. K.C.A. Chan, Chiu, and Lo report serving as directors for Cirina and holding equity in, receiving grant support from, and receiving royalty payments for patents on techniques for analysis of plasma nucleic acids for nonprenatal testing from the company, holding equity in Grail, receiving royalty payments for patents on techniques for prenatal testing from Illumina, receiving grant support and royalty payments for patents on techniques for prenatal testing from Sequenom, and serving as directors, holding equity in, and receiving royalty payments for patents on techniques for prenatal testing from Xcelom; Drs. K.C.A. Chan and Chiu, receiving consulting fees from Xcelom; Drs. K.C.A. Chan, Lam, Chiu, and Lo, holding a provisional patent for "Diagnostic applications using nucleic acids fragments" (US 62/450,541 and US 62/507,154); Drs. K.C.A. Chan, Chiu and Lo, having provisional patents for "Methods and systems for tumor detection" (US 62/411,929) and holding of more than 400 patents in different jurisdictions on techniques for prenatal testing and nonprenatal testing; Drs. K.C.A. Chan and Lo, holding patents for "Methods and kits for diagnosis, prognosis, or monitoring of Epstein-Barr virus (EBV)–associated cancer" (US7842482 and CN101622362B); Dr. Hui, receiving fees for serving on an advisory board for Bristol-Myers Squibb and Merck Sharp & Dohme; Drs. Chiu and Lo, receiving consulting fees from Sequenom; and Dr. Lo, serving as scientific cofounder and a member of the scientific advisory board for Grail, receiving fees for serving as an advisor for Decheng Capital, and holding patents for "Circulating Epstein–Barr virus DNA in the serum of patients with gastric carcinoma" (US6753137 and CN1272448). No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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