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Letter

Response to: "Study results and related evidence do not support use of HPV16 L1 DRH1 antibodies as a cancer screening test"

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We value all comments on our research, and the opportunity to provide further information on our study describing the HPV16-specific tumour marker DRH1 [1].

We used an assay that was CE certified under the European In-Vitro Diagnostic Devices Directive, implying compliance with regulatory requirements on documentation and technical validation. This includes assessments of test reproducibility. Significant bias from batch effects can therefore be excluded.

Specificity of the assay was assessed in a large control population (n = 1064*, see Table 1) of randomly selected, CRP-negative (<1 mg/l), HIV- and hepatitis-negative blood donors without history of cancer and of unknown HPV-vaccination status. Controls and cases were recruited within the same time frame and geographical region (German-speaking countries). A broad range of ages was tested, with the largest proportion comprised of 30–70-year-olds to reflect the age group most relevant for early cancer detection (Fig. 1a). Separate specificity analyses were presented for subjects below and above 30 years, reflecting official recommendations on age for HPV testing in the context of cervical cancer. Contrary to the statement in the letter [3], ROC analysis was performed on the full sample. No subjects were excluded post-hoc and all analyses were subject to approval by an independent statistician as part of the review process. Following the Letter authors' comments, we have repeated ROC analysis after

Table 1
Overview of serological test results by clinical diagnosis.

		Clinical diagnosis (HPV16-induced OPC) positive	negative*
Serological test (DRH1 at cut-off 1000 ng/ml)	positive	19	25
	negative	1	1039

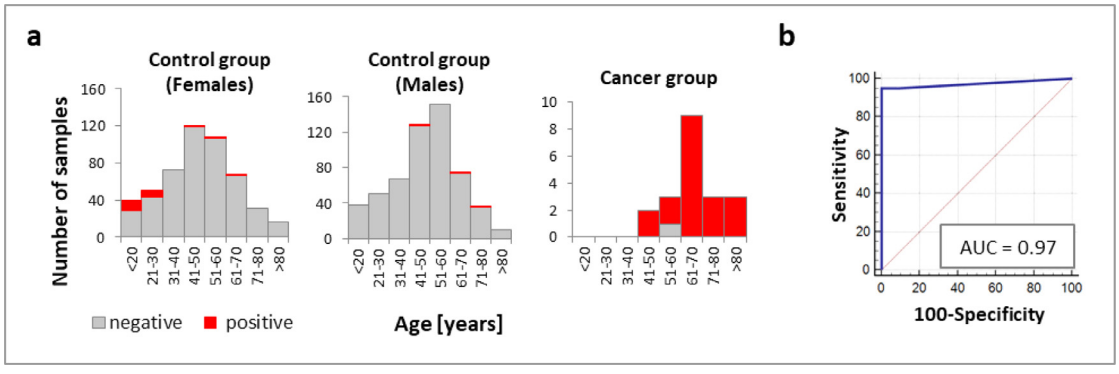

Fig. 1. a: Age- and sex-distributions of control population (n = 1064) and cancer group (n = 20). Red indicates a positive test result (HPV16-L1 DRH1 antibodies ≥1000 ng/ml). HPV vaccination status of the control population was not known, although nineteen of 25 positive results (76%) were observed in females <30 years, corresponding to the group most likely to be vaccinated.

Fig. 1.b: Receiver-operating-characteristic analysis (ROC) for the serological detection of oropharyngeal cancer using a sex- and age-matched control group (n = 260) reveals improved performance (AUC 0.97, 95% CI 0.91, 1.0) compared to original analysis.

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Table 2
Estimated impact of HPV-induced (pre)-cancer rates and DRH1 marker sensitivity and specificity on screening characteristics[a].

Targeted cases	Incidence per 100,000[b]	HPV16-attributable incidence per 100,000	Marker sensitivity	Marker specificity	Detected cases per 100,000 screened	False positives per 100,000 screened	Estimated PPV	Number to screen to detect 1 case
Oropharynx and anogenital[c] cancers	20	10	90%	99.5%	9	491	2%	11,111
Oropharynx, anogenital cancers, and CIN2+[d]	323	161	90%	99.5%	145	355[e]	29%	690

[a] detected cases, false positives, estimated PPV and number to screen have been calculated as described in Waterboer et al., letter to the editor, EBioMedicine [3].
[b] based on published data by the HPV Information Centre and the German guidelines on cervical cancer prevention [4].
[c] anogenital cancer sites include: cervix, anus, penis, vulva, vagina.
[d] CIN2+, cervical intraepithelial neoplasia of grades 2 or higher [4]. Cases of pre-cancerous lesions at other anogenital sites have not been included due to lack of representative incidence data.
[e] HPV16-induced pre-cancerous lesions of the anus, penis, vagina, vulva and OPC have not been accounted for in the incidence rate due to lack of representative incidence data and will therefore appear as "false positive" test results.

sex- and age-matching controls, without any detriment to assay performance (AUC 0.97, 95% CI 0.91, 1.0; Fig. 1b).

Although HPV status of the control population was not known, seroprevalence was greatest in females <30 years, which corresponds to the age group where we would expect to find HPV vaccinees. Further research is currently underway to distinguish vaccinated from non-vaccinated individuals. It would be inappropriate to consider the DRH1 assay for screening of HPV16-induced cancer or cancer recurrence in HPV-vaccinated individuals. It is unclear why the Letter authors should suggest that it does.

The Letter authors refer to 'natural HPV infection', conflating latent or subclinical infection with clinically relevant HPV infection. We recognise past difficulties in discriminating these cases, and our findings are significant because they show the potential for HPV16-specific DRH1 tumour marker to achieve such discrimination.

Finally, we believe that the Letter authors underestimate the potential significance of DRH1 by reducing it to its role in oropharyngeal cancer and overlooking our demonstration of assay performance in the early detection of anal cancer, with 90% sensitivity reported in the year prior to diagnosis. Further data from on-going studies suggest that similar sensitivities are to be expected for pre-cancerous anal and cervical lesions [2]. We disagree that cases of clinically relevant HPV-induced disease should be conflated with 'false positive' results, as the Letter authors have. We therefore provide updated calculations of screening characteristics, incorporating known incidence of HPV16-induced cancer and, where available, pre-cancer at both oropharyngeal and anogenital sites (Table 2).

We stated that the assay is a promising tool as a post-treatment biomarker as well as for secondary prevention purposes. Based on our data, we are fully aware that not all question are answered and further studies are needed to underpin the results of our work.

We believe that a collaborative approach would be beneficial for the field moving forward. There would be particular benefit in evaluating the DRH1 assay alongside the E6-based assay in a well-characterised study population.

When, how, and for which patient groups these findings are moved into clinical practice is a question that needs to be considered in dialogue with a wide range of disciplines, including physicians, payers, and policymakers.

Author contributions

All authors contributed equally to this work.

Declaration of Competing Interest

All authors declare no conflict of interest related to this work.

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