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LETTER TO THE EDITOR

Very early cytological and DNA-cytometric diagnosis of *in situ* carcinoma in an immunosuppressed liver transplant recipient

The recent publication in this journal by Maraki *et al.* (1) needs some critical comments. The case described was apparently already included in the series published by Maraki *et al.* (2) and thus is only a follow-up. Only indirect reference to this is given. The supposed early diagnosis of carcinoma by DNA-cytometry may be overestimated by a reader not aware of these details and metaanalysis of the results will be affected.

Secondly, the discrepancies of the initial diagnosis by the pathologists on the incisional biopsy of course are of central importance for the assessment and should have been further outlined and documented. What does 'mainly mild' (text) or 'mild to severe dysplasia' (Fig. 4) mean? Probably mild dysplasia with focal severe dysplasia? One would like to have some kind of statement like 'a panel of four pathologists reached different assessements, e.g. two pathologists found mild, one found severe dysplasia, one was undecided (or whatever the consenses was)'. Figure 4 shows dysplasia, though not classifiable from the present photograph but probably more than mild. The cytological specimen definitely shows severe dysplasia. Thus total excision was clearly indicated. What additional information important for further clinical procedure was obtained by DNA analysis? Finally, the anecdotal case reports by Maraki et al. (1,2) with the demonstration of aneuploidy 1–32 months before histology do not in any way prove the value of DNA-cytophotometry for diagnosis of cancer or precancerous lesions in the oral cavity because the question is not answered how often no dysplasia or carcinoma develop after aneuploidy has been found. No one ever doubted that aneuploid cells may be cancerous or precancerous cells. Likewise it would be interesting if aneuploidy was ever found in apparently normal cell, thus giving an indication of malignant potential not recognized in histology or cytology.

The only conclusion that can be drawn from these case reports is, that due to sampling errors incisional biopsy of suspicious lesions is not reliable and that the procedure should have been total excision right from the start. It may be doubtful that in the case presented with a highly suspicious clinical picture at a site of high risk, a cytological examination was indicated at all; total excision of the small-circumscribed lesion would have been possible and not more hazardous to the patient than the incisional biopsy. The failure of one method (histology), which is not properly performed may not prove the value of another method (DNA-cytophotometry).

Thus, the reports show that a representative cytological specimen and a good cytopathologist are preferable to a clinician obtaining poor specimens or a histopathologist forced to make a diagnosis on inadequately sampled material.

Finally, it should be pointed out that the application of DNA-cytophotometry on oral lesions mainly rests on the published findings of the group of the Norwegian scientist J. Sudbo (also cited by Maraki et al. as the basis of their work and reference). Some scientific publications of this group have been proven to be falsified, their data on DNA-cytometry should be critically reviewed under these circumstances. The value of DNA-cytophotometry for oral diagnosis should be re-evaluated also in view of the fact that after years of fruitless application it has been virtually abandoned in the diagnosis of cervical cancer and pre-cancer (pap-screening).

Prof. Dr. A. Burkhardt

References

1. Maraki D, Hengge UR, Becker J, Boecking A. Very early cytological and DNA-cytometric diagnosis of *in situ* carcinoma in an immunosuppressed liver transplant recipient. *J Oral Pathol Med* 2006; **35**: 58–60.
2. Maraki D, Becker J, Boecking A. Cytologic and DNA-cytometric very early diagnosis of oral cancer. *J Oral Pathol Med* 2004; **33**: 398–404.

Reply to Letter to the Editor

We hereby respond to the above mentioned letter concerning our case report by Maraki et al. (1).

1 The same patient had indeed already been mentioned in the publication by Maraki et al. (2). Yet, at that time the final histological diagnosis of a large oral *in situ* squamous cell carcinoma that has now been communicated in our case report, was not yet known. We have mentioned in our case report that this case had been included in the aforementioned publication. That had also been communicated to the editor of the *Journal of Oral Pathology and Medicine* in advance and his written agreement had been obtained.

2 As four different pathologists rendered three different histological diagnoses on the same sections independently from each other (one mild, two moderate and one severe dysplasia), the rationale for DNA-cytometry was to objectively clarify the presence or absence of malignant cells. This goal has been successfully achieved as this method correctly stated the presence of oral cancer cells. But no consensus could be reached among the pathologists on the grade of the supposed dysplasia.

The first written diagnosis of a mild dysplasia obtained was declared as the 'main' one.

With '*mild to severe*' we meant mild, moderate and severe.

We doubt that the grade of squamous dysplasia can be reliably judged from a printed colour image, as that on p. 59 of our criticised publication.

The dysplastic lesion was not immediately resected, as the liver transplantation was deemed more urgent by the surgeons.

3 Remmerbach et al. (3) have documented a positive predictive value of 100% for the subsequent histological finding of oral squamous cell carcinoma in 181 patients, 56 with cancer, if DNA-aneuploidy was detected in an oral smear. This article has been cited in our report. He later reported on four cases in which DNA-aneuploidy detected in oral smears preceeded the histological diagnosis of cancer by up to 15 months (4).

Grote et al. (5) also reported a positive predictive value of 100% for histologically proven cervical cancer on 274 smears with mild or moderate dysplasias after a mean follow-up of 3 months if DNA-stemline-aneuploidy was detected.

DNA-aneuploidy has so far never been found in normal, only in dysplastic or otherwise abnormal oral cells. This has been repeatedly documented by Remmerbach et al. (3, 6). But DNA-polyploidy, that is not aneuploidy may occur in lichen planus (7), after local radiation, chemotherapy (8) and epithelial regeneration.

4 Neither the insufficient reproducibility of subjective histological grading of oral dysplasias that has repeatedly been documented (9–11) nor its limited predictive value for the subsequent occurrence of oral cancer are because of insufficient sampling. Wider excisions and more representative histological diagnoses will not solve the problem of limited reproducibility, which is inherent to all subjective grading systems.

The proposal of a '*total excision*' of the reported lesion is not adequate, as its clinical appearance was rather unsuspicious, the patient suffered from haemorrhagic diathesis and the liver-transplantation was estimated priority number one.

We disagree, that the patient presented a 'highly suspicious clinical picture', nor that it was a '*small circumscribed lesion*', nor that an excision would have been '*not more hazardous to the patient*' because of his comorbidities.

It is not true, that histology was '*not properly performed*'. Instead, the discrepant histological diagnoses were because of the well known limited reproducibility of subjective histological grading of dysplasias. It is not true, that the 'clinician obtained a poor specimen and that the histopathologist made a diagnosis on inadequately sampled material'. The size of the initial biopsy was 1×2 cm.

Even smears taken from normal looking mucosa surroundig the lesion revealed DNA-aneuploidy; this has been mentioned in our report. Meanwhile the oral and maxillofacial surgeons resected a larger part of the lateral border of the tongue in which an *in situ* carcinoma was finally found by histopathology that reached the resection margins.

It is not true that 'the application of DNA-cytometry on oral lesions mainly rests on the published findings of the group around the Norwegian scientist Jon Sodbø'. Our respective publications on that subject appeared earlier and independent from that group (1–3, 4, 6, 12). Whereas Sodbø et al. reported on DNA-measurements, performed only retrospectively on archived tissue biopsies, we have only documented results obtained prospectively on oral smears from patients for which we also had clinical and mostly histological follow-up data.

The method of Diagnostic-DNA-Image-Cytometry has been recommended and standardized by the European Society for Analytical Cellular Pathology, ESACP (13–16).

It is not correct 'that after years of fruitless applications it has been virtually abandoned in the diagnosis of cervical cancer and pre-cancer'. Indeed 40 laboratories in Germany currently perform DNA-image-cytometry as a diagnostic routine procedure for the evaluation of cervical dysplasias. This procedure is reimbursed by all German health insurance companies. The list of laboratories can be found in the website http://www.sanfte-krebsdiagnostik.de: in the chapter: '3.5 Welcher Arzt macht was?'. Among the users of this method are five German university institutes of pathology. In China screening for cervical cancer on smears is currently performed in 20 early cancer detection centres applying fully automated DNA-cytometry (17). The screening-machines apply those diagnostic algorithms that we have developed and published (18).

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References

1. Maraki D, Hengge UR, Becker J., Boecking A. Very early cytological and DNA-cytometric diagnosis of *in situ* carcinoma in an immunosuppressed liver transplant recipient. *J Oral Pathol Med* 2006; **35**: 58–60.
2. Maraki D, Becker J, Boecking A. Cytologic and DNA-cytometric very early diagnosis of oral cancer. *J Oral Pathol Med* 2004; **33**: 398–404.
3. Remmerbach TW, Weidenbach H, Pomjanski N, et al. Cytologic and DNA-cytometric early diagnosis of oral cancer. *Anal Cell Pathol* 2001; **33**: 211–21.

4. Remmerbach TW, Weidenbach H, Hemprich A, et al. Earliest detection of oral cancer using non-invasive brush biopsy including DNA-image-cytometry: report on four cases. *Anal Cell Pathol* 2003; **25**: 159–66.
5. Grote HJ, Nguyen HVQ, Leick AG. Identification of progressive cervical epithelial cell abnormalities using DNA-image cytometry. *Cancer Cytopathol* 2005; **25**: 373–9.
6. Remmerbach TW, Mathes SN, Weidenbach H, et al. Nichtinvasive Bürstenbiopsie als innovative Methode in der Früherkennung des Mundhöhlenkarzinoms. *Mund Kiefer Gesichts Chir* 2004; **8**: 229–36.
7. Biesterfeld S, Füzesi L, Härle F, et al. DNA-cytometric detection of euploid polyploidization in oral lichen ruber planus. *Anal Quant Cytol Histol* 1991; **13**: 7–10.
8. Cartsburg O, Kallen C, Hillenkamp J, et al. Topical mitomycin C and radiation induce conjunctical DNA-polyploidy. *Anal Cell Pathol* 2001; **23**: 65–74.
9. Abbey LM, Kaugars GE, Gunsolley JC, et al. Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasias. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **80**: 188–91.
10. Karabulut A, Reibel J, Therkildsen MH, et al. Observer vaiability in the histologic assessment of oral premalignant lesions. *J Oral Pathol Med* 1995; **24**: 198–200.
11. Abbey LM, Kaugars GE, Gunsolley JC, et al. The effect of clinical information on the histopathologic diagnosis of oral epithelial dysplasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; **85**: 74–7.
12. Knüchel R, Mittermayer C, Böcking A. Tumorvorstadien der Mundschleimhaut – Mögliche Identifizierung obligater Präkanzerosen. *HNO* 1986; **34**: 223–8.
13. Böcking A, Giroud F, Reith A. Consensus report of the ESACP task force on standardization of diagnostic DNA-image cytometry. *Anal Cell Pathol* 1995; **8**: 67–74.
14. Haroske G, Giroud F, Reith A, et al. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part I: Basic recommendations for preparation, measurement and interpretation. *Anal Cell Pathol* 1998; **17**: 189–200.
15. Haroske G, Baak J, Danielsen H, et al. Fourth updated ESACP consensus report on diagnostic DNA image cytometry. *Anal Cell Pathol* 2001; **23**: 89–95.
16. Giroud F, Haroske G, Reith A, et al. ESACP consensus report on diagnostic DNA image cytometry. Part II: Specific recommendations for quality assurance. *Anal Cell Pathol* 1998; **17**: 201–8.
17. Sun XRS, Wang J, Garner D, et al. Detection of cervical cancer and high grade neoplastic lesions by a combination of liquid-based sampling preparation and DNA measurements using automated image cytometry. *Cell Oncol* 2005; **27**: 33–41.
18. Böcking A, Adler CP, Common HH, et al. Algorithm for a DNA-cytophotometric diagnosis and grading of malignancy. *Anal Quant Cytol* 1984; **6**: 1–8.