

Development of a Non-invasive Screening Method for Early Diagnosis of Preneoplastic Lesions

R.H. Brakenhoff¹, A.P. Graveland¹, T. Wu¹, A. Brink¹, J. Soulier², E. Gluckman², F. Pascal³, E. Velleuer⁴, R. Dietrich⁵, C.R. Leemans¹

¹VU University Medical Center, Amsterdam, The Netherlands; ²Hospital Saint Louis, Paris, France; ³Hospital Saint Louis, Paris, France; ⁴Heinrich Heine University, Dusseldorf, Germany; ⁵FA support group, Unna, Germany

Objective: Fanconi anemia (FA) patients are at high risk to develop squamous cell carcinomas (SCC). Specific risk factors for FA-SCC are bone marrow transplantation (BMT) and graft versus host disease. FA-SCC tumors seem genetically comparable to sporadic SCC tumors. The role of the human papillomavirus in FA-SCC is far from clear and seems to depend strongly on geographical differences in the studied patient groups. Recent investigations indicated that SCCs are preceded by large preneoplastic fields, and in the oral cavity these can present as white (leukoplakia) or red (erythroplakia) areas in the mucosa, macroscopical changes that can also be seen in FA patients. The majority of these fields, however, are not visible to the naked eye, but only by genetic markers and some by histology. Based on the known patterns of genetic alterations in the precursor fields, we have developed and validated a genetic assay that allows non-invasive screening of small brushed samples of the oral cavity.

Methods: After informed consent the oral cavity of 59 FA patients (43 nonBMT, 16 BMT) was brushed at 7 defined regions and all clinically visible lesions. Using 12 microsatellite markers the presence of genetic changes was determined in these samples. Twenty noncancer controls at young age were enrolled as negative controls, and 25 sporadic leukoplakia patients as positive controls with genetic analysis of the biopsies as gold standard.

Results: In none of 20 negative controls a preneoplastic field was detected suggesting a high specificity of the assay (>95%). In 9/25 sporadic leukoplakia patients the visible lesion contained genetically altered cells. In 6/7 cases analyzed the genetic changes in the biopsy were detected in the brushed samples, suggesting a sensitivity of the noninvasive assay of 80-90%. In nonBMT-FA patients we detected genetic changes in one or more samples of 20/43 cases tested, a very high frequency. In BMT-FA patients we were not able to score for genetic changes. It appeared that most samples of the 16 BMT-FA patients tested showed complex genetic patterns, suggesting that the mucosal epithelium consists of a mixture of patient and donor cells. The percentage donor cells varied from 0-100%. The same was found in a biopsy.

Conclusions: A non-invasive assay to detect genetic changes seems feasible, but needs to be optimized further. The current assay is not applicable to BMT-FA patients due to the frequent presence of donor DNA in the mucosal samples, and it needs to be adapted.

Translational Applicability: A noninvasive genetic assay might be used for early diagnosis of preneoplastic changes. When the mucosa in BMT patients is indeed taken over in part by donor cells, this phenomenon might be exploited for treatment of such lesions.