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Perspectives for colorectal cancer screening based on new molecular markers in stool

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Colorectal cancer (CRC) is the third most common malignancy in the world. Due to its slow development from endoscopically removable precancerous lesions and from surgically curable early stages, screening for CRC provides the opportunity to reduce both morbidity and mortality of the disease. To realize these goals appropriate screening methods are needed. In contrast to invasive screening methods stool testing appears to be particularly advantageous with respect to achievable compliance rates and practicalness. However, the most widely used stool test, fecal occult blood testing, has important limitations given its low sensitivity to detect precancerous or cancerous lesions. Therefore, new stool tests based on molecular markers intended to detect exfoliated neoplastic colon cells or cell products in stool are developed at present. The aim of this project was to estimate the use and to assess the suitability of these new tests for a population-based screening setting.

In a first step, a simulation model was developed which allows to estimate and to compare the potential of screening based on stool tests with various performance characteristics on the one hand, and of primary screening colonoscopy on the other hand, for the early detection or prevention of CRC. Model outcomes were calculated for the offer of annual stool testing from age 55 to 74 in combination with colonoscopic follow-up of positive test results and for the offer of screening colonoscopy as a primary screening tool at ages 55 and 65. The long-lasting risk reduction of colonoscopy allowing the removal of precancerous lesions was taken into account quantitatively. For a variety of stool tests with different performance characteristics the proportion of CRC cases early detected or prevented was estimated to be higher for stool testing in combination with colonoscopic follow-up of positive test results compared to primary screening colonoscopy assuming levels of compliance to be expected for the respective screening scheme. Optimizing performance characteristics of stool tests in terms of

detecting precancerous lesions in addition to those in terms of detecting CRC appeared to be crucial for maximizing effectiveness of CRC screening based on stool tests.

Furthermore, a comprehensive literature review was performed summarizing current evidence for new stool tests aimed at detecting colorectal neoplasms under screening conditions. Special attention was given to aspects being of prime importance for a successful population-based screening setting, e.g. sensitivity for early stages, sensitivity for adenomas and practicalness. MEDLINE database was searched for relevant articles published until May 2005. Studies were included if they comprised more than 10 cases and more than 10 controls. Details on the study population, on performance characteristics and on the stool collection procedure were extracted from published reports. Overall, 34 studies, mostly retrospective, were included investigating 20 different stool markers or marker combinations. The study populations were very heterogeneous and mostly very small. About half of the studies reported sensitivity for adenomas in addition to sensitivity for CRC, and less than half of the studies reported sensitivity by tumor stage. Performance characteristics of stool tests varied to a large extent. For most DNA-based markers specificity was about 95% or higher, but sensitivity was mostly low even for invasive CRC. More studies with larger sample sizes were done for protein-based markers, which typically had lower specificity, but first results regarding sensitivity are promising. In most studies, stool samples were frozen within a rather short time period after defecation. While promising performance characteristics have been reported for some tests, more pervasive evidence from larger, prospectively designed studies is needed which also consider aspects of practicalness, e.g. the possibility of mailing the samples.

Finally, stool samples from older adults participating in the ESTHER study, a large population-based cohort study, were analyzed for both a DNA-based stool marker (*K-ras*) and a protein-based stool marker (tumor M2-pyruvate kinase). Mutant *K-ras* in stool was analyzed among 875 study participants. With about 8%, the prevalence of this marker was higher than expected. Stool samples from participants who were diagnosed with advanced neoplasia 1-2 years after sample collection all tested negative for mutant *K-ras*. Fecal tumor M2-pyruvate kinase concentrations were determined among 916 participants of the ESTHER study and among 65 separately recruited colorectal cancer patients. At a cut-off value of 4 U/ml, overall sensitivity for CRC was 68%. Sensitivity was higher for colon than for rectum cancer and was higher for advanced than for non-advanced stages. Specificity was estimated to be about 73-79%. Stability testing showed degradation of this marker at room temperature which may

hamper sample mailing in the screening setting. For both markers, methodological aspects relevant to large scale application require further examination.

Overall, the high potential of new stool tests for reducing the burden of CRC could be confirmed in theory. Development and adequate evaluation of stool tests which might finally prove suitable and (cost-) effective in a population-based screening setting remains the challenge of future research.