Colorectal cancer (CRC) is the third most common form of cancer worldwide and accounts for a significant proportion of cancer death. Although high incidence rates of this tumor are associated with Western lifestyle and diet, the incidence in other parts of the world is also not negligible and rising [1]. Due to high incidence rates that are associated with significant morbidity and mortality, CRC represents a major public health issue in the European Union (EU), US as well as many other countries around the globe.

Considerable progress that has been achieved in the management of CRC during the past two decades encompassed improvements in diagnosis, surgical techniques as well as non-surgical therapies including chemotherapy, targeted treatments and radiotherapy. The progress accomplished during the past quarter of the century is perhaps most visible in the management of metastatic CRC. Twenty years ago systemic therapy was limited to different regimens of fluoropyrimidines and the only possible “target” therapy was the administration of chemotherapy as hepatic arterial infusion [2]. The pharmacologic treatment has evolved since the late 1990s in small steps that together signified a big leap in the therapy of metastatic CRC. The discovery of the activity of oxaliplatin and irinotecan in metastatic CRC not only provided first truly effective second-line treatment options in metastatic CRC patients failing fluoropyrimidines, but also the combinations of oxaliplatin or irinotecan with fluoropyrimidine backbone regimens have soon demonstrated superior activity in the first-line setting and quickly became the standard of care [3]. Subsequent advent of truly targeted treatments aiming at either vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) further changed the landscape of the treatment of metastatic CRC [4, 5]. Thus, despite the relative paucity (compared to other cancers) of active agents, the available agents, including fluoropyrimidine (mostly 5-fluorouracil or capecitabine), oxaliplatin, irinotecan, anti-VEGF drugs bevacizumab, aflibercept and regorafenib, or anti-EGFR antibodies cetuximab and panitumumab may be combined in multiple lines of therapy providing disease control lasting often for years.

In spite of all this progress, advanced or metastatic CRC remains, for the majority of patients, an incurable disease. The cure rates are much higher in patients with early stage tumors, and most patients presenting with stage I or stage II can be treated by surgery alone. Unfortunately, a significant proportion of the newly diagnosed patients presents with stage III or stage IV (metastatic) disease. Thus, early detection of CRC is of great importance, specifically in countries with high incidence rates, including US and EU. The potential impact of precocious detection on the mortality from CRC is expected to be more pronounced compared to the potential of new therapies for advanced disease, and early detection is also potentially more cost-effective.

Similarly to tumors of other primary locations [6], laboratory medicine plays an increasingly important role during all phases of management of patient with CRC, including screening, diagnosis, treatment and follow-up after therapy. Without the contribution of laboratory medicine, current standard therapy would not be possible. For example, testing for tumor RAS mutations constitutes an integral part of therapeutic algorithm in patients with metastatic CRC as it identifies population of patients in whom the administration of antibodies targeting EGFR will not be helpful and may even be harmful [7–9]. Despite all progress the mortality for CRC is still rather high, and efforts aiming to improve outcomes are directed not only at the therapy, but also at an early identification of the disease through screening programs [10].

Randomized, controlled trials have shown that the implementation of annual or biennial fecal occult blood tests (FOBTs) is associated with a 15%–33% decrease in CRC mortality rate [11–13]. However, a body of evidence demonstrates that FOBTs only detect approximately 13%–50% of cancer with one round of screening in asymptomatic patients [14, 15]. A recently published article on the diagnostic performance of FOBT under routine screening...
conditions also demonstrated that FOBTs are expected to miss approximately nine out of 10 advanced adenomas, as well as three out of four cancers [16]. A review and meta-analysis of studies on diagnostic sensitivity and specificity of FOBTs published in 2010 yielded a summary estimate of 0.36 (95% CI 0.24–0.47) sensitivity and 0.96% (95% CI 0.94%–0.97%) specificity for detecting CRC when only those studies that attempted to correct for verification bias (i.e., those aimed for confirmation of absence of CRC in all FOBT–negative subjects) were taken into account [17]. The results described in the paper of Brenner et al., which demonstrate poorer performance of FOBTs in routine application compared with previously reported trials, appear plausible, given that quality assurance and training are expected to be less rigorous in routine settings than under controlled study [16]. Moreover, the cut-off concentration of FOBTs is typically defined by the manufacturers and cannot easily be adjusted by end-users. Finally, adherence to FOBT in real world screening programs is reportedly low, raising further concern about its real effectiveness as a screening test [18]. The more recently developed fecal immunochemical tests (FITs) confer substantial benefits over FOBT, since: 1) they are human-hemoglobin-specific; 2) they have superior diagnostic sensitivity and specificity for detecting CRC when compared to FOBT [19]; 3) they do not require dietary or medication restriction, thus improving patient adherence to screening programs; and 4) they allow end-users to validate and eventually modify the cut-off defined by the manufacturer in the quantitative version. For all these reasons, the performance of FITs is now recommended in current guidelines [20].

However, as for many other laboratory tests, all FITs are not equal and information has been gathered not only to show that available FITs are characterized by different technical features, but also that several variables should be considered when adopting these tests within screening programs [21]. In a recently published Editorial in Clinical Chemistry and Laboratory Medicine, Fraser and colleagues [22] emphasized that all aspects and performance characteristics of FITs should be carefully evaluated, by adoption of the recently published Faecal Immunochemical Test for Haemoglobin Evaluation Reporting (FITTER) standards and checklist [23]. Along with analytical characteristics and quality specifications (e.g., quantitative but not qualitative assays allow the evaluation and setting of the most accurate cut-off), essential pre-analytical and post-analytical issues should also be weighted before implementation of screening programs based on FITs.

In this issue of Clinical Chemistry and Laboratory Medicine, two companion papers provide further insights in this area of research. The first article explores the pre-analytical phase of fecal blood assay and, in particular, the stability of hemoglobin under different storage conditions [24]. Concern has been raised regarding delayed sample delivery, since the globin component undergoes rapid degradation while the hem component is subjected to a slower catabolic pathway, involving processes that are still partially elucidated but are thought to include the removal of iron by colonic bacteria. The results of the study show that: 1) fecal samples behave differently from hemoglobin solutions, since no degradation occurs in synthetic hemoglobin samples, thus emphasizing that stability testing should always be performed with real fecal samples; 2) a new device, which uses a buffer containing an appropriate stabilizing agent, allows improved sample stability at both 4 °C and room temperature; this device is hence more suitable for screening procedures, in which delivery time and storage conditions are particularly challenging; 3) with the “old” collection device, hemoglobin degradation generated false-negative test results in samples with hemoglobin concentrations close to the analytical cut-off, thus explaining the false-negative rate that has been previously reported in a higher number of patients with minor adenomas than in those with advanced adenomas [25].

The second article explores the final part of the total testing loop, the so-called post-analytical phase. It is known that fecal Hb (f-Hb) do vary with sex and age, being higher in men and in the elderly [26]. However, the paper by Fraser and colleagues demonstrates that the degree of variation seems inconsistent across countries, so that data on f-Hb may be scarcely transferable across geography, and any single cut-off is expected to be associated with different outcomes in the different countries [27]. This article, therefore, supports the view that a single f-Hb cut-off in any CRC screening program is far from ideal. The authors pointed out that “individualisation of CRC screening should be the optimum approach with f-Hb in an individual, alone or with other important factors such as sex and age”.

In addition to early diagnosis, the estimation of the individual patient prognosis plays a crucial role in the management of each individual patient diagnosed with CRC, especially in patients with early disease. The majority of patients with early (stage I or stage II) CRC are cured with surgery alone, but an important minority of patients will experience disease recurrence. Preventive measures aiming to avoid later recurrence including chemotherapy or, in the case of rectal carcinoma, radiotherapy are associated with significant cost and, more importantly, morbidity and risk of complications. Thus, biomarkers that would help to identify patients at high risk of recurrence represent a hitherto unmet medical need in this disease.
Different biomarkers used to predict prognosis included circulating tumor biomarkers or biomarkers associated with host response to neoplasia. The paper by Rapti et al. [28] analyzes the prognostic significance of microRNA miR-182 in CRC patients. The authors observed higher miR-182 quantity in tumor tissue compared to normal colorectal mucosa. Moreover, miR-182 expression increased with higher grade, stage, tumor invasion and lymph node involvement. Most importantly, high miR-182 concentrations were associated with poor prognosis.

The study by Rapti et al. [28] still constitutes an early stage of biomarker development. A number of studies have established the prognostic significance of microRNAs in tumors of different primary locations [29]. The diagnosis of malignant disease is established by histological examination, and differences in the quantity of microRNAs are not so important from the perspective of diagnosis. However, as outlined above, the estimation of prognosis is of great importance in setting the strategy of the patient management. From a point of view of general practice, circulating biomarkers are certainly more useful and less invasive than biomarkers that are determined in tumor tissue. Circulating biomarkers may also more easily be assessed repeatedly, allowing not only for the estimation of prognosis at the start of therapy, but also for monitoring the effect of therapy or early detection of recurrent disease. It remains to be determined whether miR-182 will be useful in this setting.

In conclusion, at the down of the third millennium, the optimal screening strategy for CRC is still an open issue. Although evidence has been provided that molecular biology [10, 30] and analysis of methylated genes [31] represent valuable perspectives, fecal hemoglobin testing is still considered the biochemical gold standard. Once again, however, the take home lesson is that analytical quality specifications play an essential role in any laboratory test, including fecal blood testing, but many other extra analytical variables should be considered [32]. In particular, in the case of fecal blood testing, pre-analytical issues (e.g., quality of the collection device, sample handling and storage) strongly influence the diagnostic accuracy of the test, particularly in the context of a screening program. In the final part of the cycle, there is an increasing need to recognize the importance of the cut-off concentrations and to standardize the measure unit, since the expression in terms of “μg Hb/g” feces is now recommended and should be universally adopted. The value for patients should be achieved only if all steps of the total testing cycle are well identified, characterized and monitored.

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