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Biomarkers for risk stratification of neoplastic progression in Barrett esophagus

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Abstract. Barrett esophagus (BE) is caused by chronic gastroesophageal reflux and predisposes to the development of esophageal adenocarcinoma through different grades of dysplasia. Only a subset of BE patients will finally develop esophageal adenocarcinoma. The majority will therefore not benefit from an endoscopic surveillance program, based on the histological identification of dysplasia. Several studies have been performed to find additional biomarkers that can be used to detect the subgroup of patients with an increased risk of developing malignancy in BE. In this review, we will summarize the most promising tissue biomarkers, i.e. proliferation/cell cycle proteins, tumor suppressor genes, adhesion molecules, DNA ploidy status and inflammation associated markers, that can be used for risk stratification in BE, and discuss their respective clinical application.

Keywords: Barrett esophagus, biomarkers, esophageal adenocarcinoma

1. Introduction

Barrett esophagus (BE) is characterized by the replacement of the normal stratified squamous epithelium of the distal esophagus by columnar epithelium with specialized intestinal metaplasia (IM) [77], which is characterized by the presence of goblet cells. Chronic gastroesophageal reflux is the most important factor in the development of BE [1]. BE is a premalignant condition predisposing to the development of esophageal adenocarcinoma (EAC). This development is a gradual process in which the accumulation of (epi)genetic changes causes disruption of important biological processes at the cellular level, which can ultimately cause these cells to behave as cancer cells, i.e., invading surrounding tissues and metastasize. The morphologic counterpart of these molecular changes is called dysplasia. Dysplasia can be subclassified into two distinct morphological stages, each representing a subsequent step in tumor progression towards EAC, i.e., low-grade dysplasia (LGD) and high-grade dysplasia (HGD) [78,79].

Nowadays, morphological assessment of biopsies is the best method to assess whether and to what stage neoplasia in BE has progressed in an individual patient, and based on this, to determine the interval of endoscopic surveillance in these patients. The aim of surveillance is to detect progression of dysplasia at an early and therefore likely curable stage [77].

Although EAC is frequently accompanied by Barrett's metaplasia, only approximately 5% of patients who present with EAC are known with a prior diagnosis of BE [17,19]. Moreover, the risk of developing EAC in BE is low and has been suggested to be approximately 0.5% on a yearly basis [16,20,31]. Therefore, the majority of patients with BE will not benefit from an endoscopic surveillance program [16,20,31]. Further stratification of the risk of progression of BE to EAC might permit more effective targeting of repeated endoscopy to patients with an increased risk of progression.

At present, patients with BE are only risk stratified by the grade of dysplasia as assessed by histological evaluation of endoscopically taken biopsies [58]. In 1988, histologic criteria for grading dysplasia were established by a group of experts in gastrointestinal pathology [72]. Histological grading according

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to these criteria is however accompanied by considerable interobserver variability, especially for the discrimination between no dysplasia (ND) and LGD [57]. Considerable effort has been put in the identification of one or more biomarkers that could distinguish patients with a high risk from those with a low risk of EAC development. A biomarker in this regard can be defined as an indicator of a pathological process. The ideal biomarker for this would probably be a molecule that shows a variation in expression that is associated with neoplastic progression and is already detectable at an early stage in this process [54]. In this review, the most promising tissue biomarkers known so far will be discussed.

2. Potential biomarkers for risk stratification

The transformation from a normal cell into a tumor cell requires several alterations, each of them leading to the induction of proteins involved in tumorigenesis or downregulation of proteins protecting the cell [76]. These alterations comprise usually genetic lesions or altered methylation patterns of genes, resulting in changes in mRNA and protein expression. The molecules involved in these processes may therefore provide markers for the detection of early malignant progression. Based on the molecular alterations these markers can be divided in different groups, which will consecutively be described in this review: proliferation/cell cycle proteins, tumor suppressor genes, adhesion molecules, DNA content, and inflammation associated markers. In Fig. 1, the pattern of expression of these biomarkers is shown in a schematic overview.

2.1. Proliferation/cell cycle proteins

Tissue damage by gastroesophageal reflux will lead to proliferation in order to replace the injured cells by new ones. In order to proliferate, a cell needs to progress from the G1 to the S phase in the cell cycle (Fig. 2). Progression to a next stage in the cell cycle requires the action of cyclin-Cdk (cyclin-dependent kinase)-complexes. When this proliferation runs out of control, neoplastic lesions will occur. Abnormalities of proteins that play a role in the progression from the G1 to the S phase can be observed during carcinogenesis. These proteins, i.e., PCNA, Ki67 and Cyclin D1 could therefore serve as biomarker in predicting the risk of neoplastic progression.

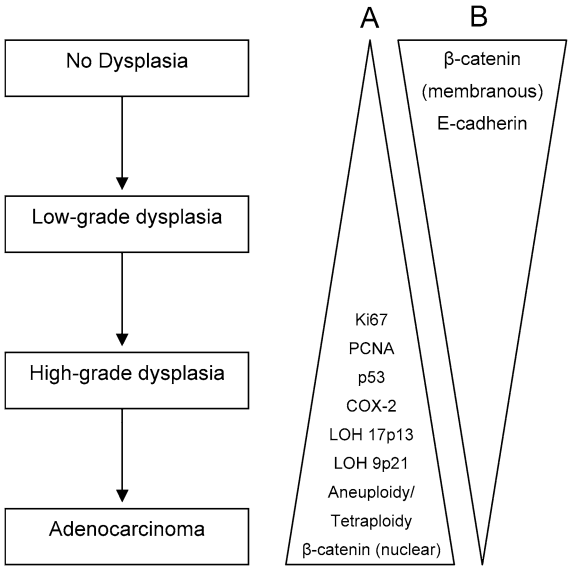

Fig. 1. Schematic overview of the expression of the discussed biomarkers in the progression from Barrett's metaplasia towards esophageal adenocarcinoma. Biomarkers are grouped for those with an increased (A) or decreased (B) expression in the metaplasia–dysplasia–carcinoma sequence.

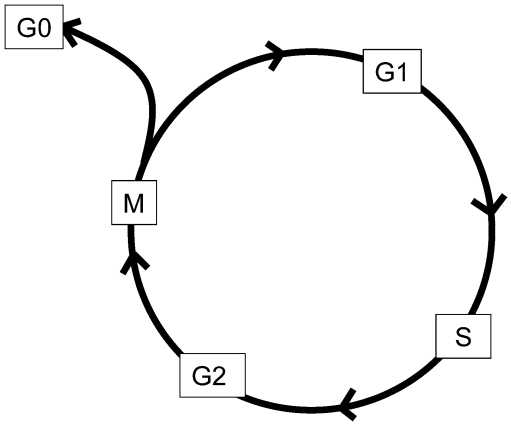

Fig. 2. Cell cycle. G1 = gap 1, cells in resting phase (DNA = 2 N); S = DNA synthesis; G2 = gap 2, cells are duplicated (DNA = 4 N); M = mitosis, cells are divided in 2 daughter cells (DNA = 2 N); G0 = resting phase, cells that cease division.

2.1.1. PCNA

Proliferating cell nuclear antigen (PCNA) is a cofactor of DNA synthase and an indicator of cell cycle progression at the G1/S transition phase in the cell cycle (Fig. 2) [8]. PCNA was the first proliferation marker that could be used for immunohistochemical staining of formalin-fixed paraffin tissue. As a consequence most of the initial proliferation marker work has focused on PCNA, also because no alternatives

were available [80]. Several studies have shown that PCNA staining is increased in HGD/EAC, with an increase in the intensity of PCNA expression with extension of the proliferative compartment upwards to the superficial layers of the glands as is seen in dysplasia [27,42,45]. This was however not confirmed in another study, in which PCNA was found to be of limited value in differentiating between ND, LGD and 'indefinite for dysplasia' (IND) in BE [43]. A disadvantage of PCNA staining is that it is affected by the fixation method of the tissue, with consequently staining of quiescent cells (G0 phase) during antigen retrieval (Fig. 2) [80]. Therefore, PCNA is probably not a reliable marker that can be used for the prediction of patients at risk of neoplastic progression in BE.

2.1.2. Ki67

The human Ki67 protein is present during all active phases of the cell cycle (G1, S, G2, M), but is absent in resting cells (G0) (Fig. 2). Although some of its features have been characterized, such as phosphorylation and nuclear transport, the exact function of the Ki67 protein is still largely unknown [80]. Expression of the Ki67 protein is strictly associated with cell proliferation. The fraction of Ki67 positive cells have been demonstrated to correlate with the clinical course of the disorder [80]. No other known protein has so far been shown to have an expression pattern that is so closely associated with the proliferative status of the cell. With the development of the Ki67 equivalent MIB-1, Ki67 immunostaining can be easily performed on formalin-fixed paraffin-embedded tissue. In contrast to PCNA (see above), the Ki67-antibody does not stain quiescent cells, making Ki67 the preferred proliferation marker (Fig. 3) [14].

The extent of immunohistochemical Ki67 expression is associated with each histological grade, showing a stepwise increase in Ki67 expression with neoplastic progression of BE [64]. In a study by Hong et al., statistical differences in expression levels between no dysplasia (ND), LGD and HGD were found. The category IND however had a great variety in expression pattern, sometimes even resembling HGD. These authors concluded therefore that Ki67 better can be used as an additional parameter to differentiate between BE patients with or without dysplasia [40]. In contrast, Olvera et al. concluded that Ki67 was able to differentiate LGD from HGD, but could not distinguish LGD from reactive changes (IND). The number of cases in this study was however small ($n = 25$), making this conclusion disputable [65]. Currently, only cross-sectional studies on Ki67 expression in BE have been reported and longitudinal follow-up studies for evaluating the value of Ki67 as biomarker for risk prediction are therefore indicated. In a study by Polkowski et al. [66], using morphometry with assessment of the percentage of nuclei positive for Ki67 per 100 counted nuclei, it was shown that Ki67 was a valuable marker to overcome difficulties with subjective grading [2].

Most studies on Ki67 expression in Barrett epithelium have been performed with immunohistochemistry. Detection of Ki67-positive cells in Barrett biopsies can also be performed with flow cytometry, making rapid quantification possible [75]. Fresh frozen biopsies are however required for flow cytometric evaluation, while immunohistochemistry can be performed on more easily available paraffin-embedded biopsies.

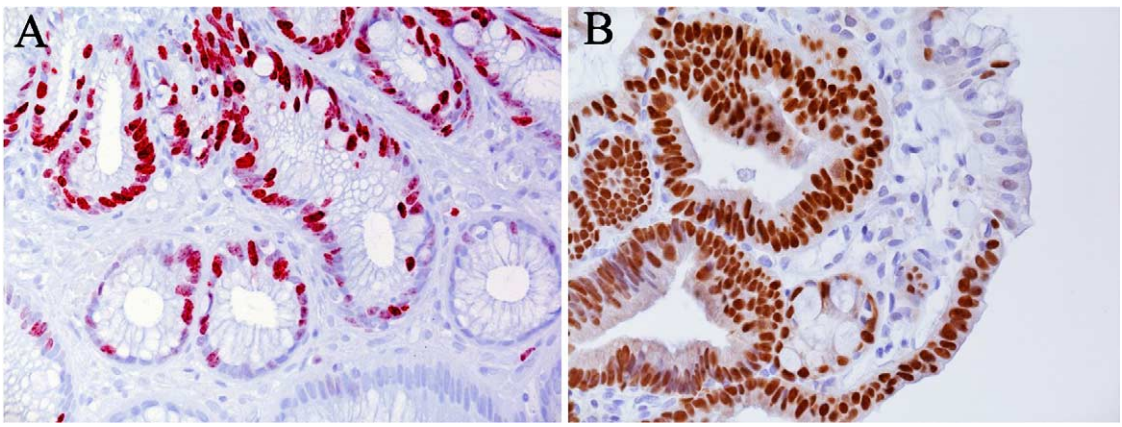

Fig. 3. Examples of immunohistochemical staining for Ki67 and p53 expression in Barrett esophagus (specialized columnar epithelium). Original magnifications ×400. (A) Ki67 overexpression; (B) p53 overexpression.

In contrast to immunohistochemistry, flow cytometry has the disadvantage that the identity of Ki67-positive cells cannot be determined. The usefulness of flow cytometry for Ki67 lies in the possibility to distinguish Ki67-positive G1 cells from quiescent G0 cells, which is important if combined with evaluation of the ploidy status (see further in 'DNA ploidy').

2.1.3. Cyclin D1

The Cyclin D1 gene is known to regulate the G1/S-checkpoint in the normal cell cycle (Fig. 2), and may therefore play a role in carcinogenesis [26]. The role of cyclin D1 in cell cycle control is mediated through cyclin D1-cyclin-dependent kinase (cdk) complexes [4]. In a prospective study by Bani-Hani et al., immunohistochemically detected cyclin D1 was found to be significantly overexpressed in 92% of samples with EAC. In addition, 67% of biopsies of these patients taken at earlier time points showed cyclin D1 overexpression, compared to 29% of biopsies of controls without malignant progression in BE. Based on these results, it was suggested that cyclin D1-staining could be a useful biomarker in identifying BE patients with an increased risk of neoplastic progression [4]. These results were however contradictory to more recent studies, in which cyclin D1 was not significantly associated with risk of malignant progression [51,61]. Additional studies are clearly warranted.

Geddert et al. found that *cyclin D1* polymorphisms in patients with EAC were not significantly different from those of healthy controls, and therefore were unlikely to be associated with an increased risk of EAC [26]. In contrast, Casson et al. found that the CCND1 A/A genotype was associated with an increased risk of developing BE and EAC, however no association was found between this genotype and cyclin D1 overexpression [13].

2.2. Tumor suppressor genes

Tumor suppressor genes control cell proliferation by preventing cells from uncontrolled expanding. Proteins that activate the tumor suppressor gene behave as tumor suppressors. In a mutated tumor suppressor gene, the function may be lost due to inactivation, and consequently the protein has become an oncogene, leading to uncontrolled growth of mutated cells, and finally to malignancy. It has been suggested that mutated tumor suppressor genes may have the ability to predict neoplastic progression. In BE, particularly the role of p53 en p16 has been explored.

2.2.1. p53

p53 is a tumor suppressor gene, located on the 17p13 chromosome. The gene is involved in controlling cell proliferation [52]. Normally, cells contain low levels of wild-type p53. Wild-type p53 regulates two common responses to oncogenic stress, i.e., cell cycle arrest/DNA repair and apoptosis. In cells that are early in the G1-phase, p53 triggers a checkpoint blocking further progression through the cell cycle, allowing the damaged DNA to be repaired before the cell enters the S-phase (Fig. 2) [56]. If the DNA damage cannot be repaired, p53 induces apoptosis [37]. This suggests that failure of p53 to respond to DNA damage will increase the susceptibility to oncogenic changes. Mutated p53 is dominant negative, as it will overwhelm the wild-type protein and prevents it from functioning [56]. These p53 mutations are associated with an increased half-life of the p53 protein, resulting in its accumulation in the cell nucleus to levels that can be detected by immunohistochemistry (Fig. 3) [39]. In contrast, wild-type p53 has a short half-life, and as a consequence these proteins do not accumulate and are therefore usually below the detection threshold of immunohistochemistry [41]. Approximately 90% of the p53 mutations are point mutations [44].

As a consequence of DNA damage, the percentage of cells in the G0/G1 or G2/M-phase that require DNA repair is increased [56]. This can be accompanied by p53 mutation and protein accumulation [56]. Several studies have shown a stepwise overexpression of p53 with increasing grades of dysplasia in BE [46,64,69,85]. Younes et al. suggested that p53 accumulation might even occur before the phenotypic changes characteristic of dysplasia and malignancy become obvious, since normal-appearing nondysplastic glands adjacent to dysplastic glands or carcinoma were also positive for p53 [90]. p53 as a biomarker of malignant progression in BE was confirmed in other studies, but the sensitivity of this marker alone in these studies was too low to predict cancer risk [4,61]. If combined with other biomarkers, such as cyclin D1, β-catenin and COX-2, p53 was also found to be of limited value [61].

Although immunohistochemistry for detecting p53 is cheap, quick, and easy to apply compared with other techniques, there are some limitations that are important to consider. The p53 antibodies that are commonly used do not only stain the mutant p53, but also detect wild-type p53. Thus, overexpression of the p53 protein does not correlate with p53 mutation *per se* [41,44]. A second limitation of p53-based immuno-

histochemistry is that mutations for this tumor suppressor gene may exist without protein overexpression. In about 30% EACs a chain-terminating mutation is found to be present, leading to a truncated p53 protein, which will not be detected by immunohistochemistry [34,41,44].

Another mechanism of inactivation of the wild-type p53 is loss of heterozygosity (LOH) for one or two alleles of the 17p13 gene [41]. LOH has been shown to occur in 0–6% of BE patients with ND, 20–27% with LGD, 57% with HGD [28,71] and 54–92% with EAC [11,30], and sometimes coexists with a p53 mutation [11,30]. It has been shown that clones of 17p13 LOH show variable expansion within the Barrett segment [25], and a larger size of the LOH clone seems to be associated with a higher risk of progression to EAC [53]. A strong association has also been found between 17p13 LOH and an abnormal flow cytometric DNA content in BE [10,24,25]. In 91% of flow cytometrically detected aneuploid/tetraploid cases, LOH at 17p13 was also present, in contrast to only 17% of diploid cases [25]. In another study by the same group, LOH at 17p13 was found in 91% of diploid cases, in which aneuploidy developed during follow-up. Thus LOH preceded the development of aneuploidy during neoplastic progression in BE [10]. Recently, these investigators showed in a prospectively followed cohort that 37% of patients with LOH at 17p13 progressed over time from ND to EAC, compared to 3% of patients without LOH, suggesting that 17p13 LOH is an early event in the neoplastic cascade of BE [74]. Since the technique for 17p13 LOH is not routinely available, it is not commonly being applied yet [22].

2.2.2. p16

p16 is a tumor suppressor gene, which is located on chromosome 9p21. This gene is also known as cyclin-dependent kinase inhibitor 2 (CDKN2), INK4, or multiple tumor suppressor 1 (MST1) [25]. Normally, the expression of p16 results in G1 arrest by inhibiting the cyclin-dependent kinases that are responsible for phosphorylation of the retinoblastoma protein (Fig. 2). Inactivation of p16 will lead to uncontrolled cell proliferation [8]. LOH is the predominant mechanism for inactivation of one of the p16 alleles, occurring in approximately 75% of samples taken from EAC [5]. Clones of cells with LOH at 9p21 have been shown to expand along the Barrett segment, creating a condition in which other mutations may arise that are able to induce EAC [25,89]. CpG island methylation, mutation or homozygous deletions have also been suggested to be responsible for inactivation of the remaining p16 allele [30,36,88,89]. Epigenetic modification of genes may already take place in normal mucosa of patients at increased risk of developing EAC, since hypermethylation was also detected in 56% of biopsies from squamous epithelium of patients with EAC [36], with no differences being found in the prevalence of p16 abnormalities (i.e. p16 CpG island methylation, p16 mutation and 9p21 LOH) with advancing grades of dysplasia (88% in ND, 87% in LGD and 86% in HGD) [89]. It was shown that both LOH at 9p21 and p16 mutation occur as early lesions in diploid cell populations, prior to the development of aneuploidy and cancer [5,25]. In a large prospective study, it was shown that the combination of 9p LOH, 17p LOH and DNA content abnormalities, provided a significant prediction of the risk of progression towards EAC [24]. Although LOH at 9p21 is a common event in BE, other large-scale studies have not been performed yet. In addition, the technique is not routinely available in most centers.

2.3. Adhesion molecules

Epithelial cells are tightly connected (cell–cell adhesion) with each other and one of the functions of this adhesion is to prevent development of malignancies by inhibition of proliferation. If cell–cell adhesion is loosened, penetration of toxic compounds, pathogenic organisms and inflammatory cells may occur which can cause DNA damage for example through the formation of oxygen radicals [62]. These oxygen radicals may induce DNA mutations, leading to carcinogenesis. In addition, the loosened cell–cell connections could make it easier for neoplastic cells to invade neighbouring tissues. Changes in adhesion proteins could therefore be valuable in predicting neoplastic progression of BE towards HGD/EAC. The most commonly reported adhesion proteins are E-cadherin and β-catenin.

2.3.1. E-cadherin and β-catenin

The transmembrane glycoprotein E-cadherin belongs to the family of calcium-dependent Wnt-related genes and plays a role in morphogenesis of tissues during embryogenesis. β-Catenin is directly linked to E-cadherin and together these proteins mediate cell-to-cell adhesion. The cell adhesion function of E-cadherin is frequently disturbed in cancer processes either by downregulation or by mutation of the E-cadherin/catenin genes [7]. Adenomatous polyposis coli (APC) tumor suppressor gene (located at 5q21) regulates intracellular concentration of β-catenin by causing its degradation. When the APC tumor suppres-

sor gene is mutated, β-catenin accumulates in the nucleus and binds to transcription factors, resulting in the promotion of cellular proliferation and the prevention of cellular death [8]. Normally, β-catenin is expressed in the membrane [9]. In BE, a decrease of both E-cadherin and membranous β-catenin on the one hand and an increase of nuclear β-catenin on the other hand has been observed during progression from BE to EAC [3,9,81,84,87]. Bani et al. reported a reduction of membranous β-catenin expression in 5% of ND, 16% of LGD, 68% of HGD and 80% of EAC [9]. In a case-control study by Murray et al., a moderate elevation of the odds ratio (OR 1.05 for focal staining and OR 2.40 for diffuse staining) was found for increased nuclear β-catenin expression in cases (EAC) compared to controls, however this elevation was not significant [61]. As a result of these contradictory findings and the absence of large scale clinical cohort studies, the practical value of these proteins as biomarkers for predicting risk of neoplastic progression in BE is still unclear.

2.4. DNA content

With the exception of germ-line cells, all other cells are normally diploid (2N). Human malignancies are associated with genomic instability, and many solid tumors show abnormalities of the cellular DNA content (aneuploidy or tetraploidy), which can be assessed by flow cytometry [63]. Duesberg et al. even proposed a new chromosomal cancer theory, in which aneuploidy is the key factor for developing cancer. In this theory, aneuploidy can generate new phenotypes, independent of mutations [18]. Aneuploidy is defined by losses or gaines of intact chromosomes or segments of chromosomes [18], and is diagnosed if an increased number of cells are in the S phase of the cell cycle (Fig. 2). This can be seen at flow cytometric analysis as a second peak at $>$2.7 N in the histogram, comprising at least 2.5% of nuclei [67,70]. Tetraploidy is present if $>$6% of the nuclei are in the G2 phase, which is expressed by an increased 4 N fraction (within a range of 3.85 N to 4.1 N) at flow cytometry [21,67,70,71]. Loss of heterozygosity (LOH) may also lead to a change of the DNA content, due to a loss of one or two alleles of a gene, leading to inactivation of a protein [41], as described above for p53 and p16. Finally, LOH can also be present without any change in DNA content, as it can arise from gene conversion, mostly through mitotic recombination [23]. Lai et al. have shown by using array-CGH (comparative genomic hybridization) that LOH in BE can occur by homologous recombination [50].

2.4.1. DNA ploidy

Neoplastic progression in BE is also associated with a process of genomic instability, leading to evolution of multiple aneuploid populations and finally to the development of a clone of cells capable of malignant invasion [68].

A correlation between an increase in the percentage of biopsies with an abnormal DNA content (aneuploidy or tetraploidy) and an increase in the grade of dysplasia in BE has been reported [47,59,75]. The percentage of abnormal DNA content ranges from 0–13% in ND, 0–60% in LGD, 40–100% in HGD and 71–100% in EAC [29,59,71,75]. Follow-up studies have suggested that the combination of histology and flow cytometry could be useful for identifying BE patients at risk of developing EAC [70,73,86]. Reid et al. reported in a prospective surveillance cohort that 9/13 patients with aneuploidy or tetraploidy developed HGD or EAC, compared to none of 49 patients with diploid cell populations [70]. In a study of Teodori et al., these results were confirmed [86]. In addition, it was found that the 5-year cumulative cancer incidence among 247 patients with ND, IND or LGD was 0% for diploid cases, compared to 28% for those with aneuploidy or tetraploidy [73]. In contrast, Gimenez et al. found that DNA content as detected by flow cytometry was not able to predict progression in patients with ND or LGD. In this study, it was suggested that in the 'indefinite for dysplasia' group, abnormal DNA content could be used to differentiate between future neoplastic progression and reactive epithelial changes [28]. Combination of DNA content abnormalities with other biomarkers, such as 17p13 LOH and 9p21 LOH, has been shown to improve the risk prediction of EAC in BE [10,24,25]. The majority of studies employing flow cytometry have been performed on fresh material [70,71, 73,75,86]. Compared with flow cytometry on formalin fixed, paraffin-embedded biopsies material, the resulting histograms on fresh material are of better quality. This is mainly due to less variability in staining and smaller amounts of debris in fresh biopsies, resulting in greater precision of measurement. A disadvantage of fresh material is however that immediate processing following biopsy is required to prevent the occurrence of false-positive DNA aneuploidy results [67]. This method is therefore not applicable in centers without an infrastructure to process fresh biopsy samples. The technique of flow cytometry has largely been improved, in a way that the results on the more easily available formalin fixed, paraffin-embedded biopsies have become comparable with those on fresh tis-

sue [29,38,59]. This suggests that DNA content as assessed by flow cytometry has the potential to become an easy to apply and useful biomarker for predicting neoplastic progression in BE. Prospective follow-up studies on formalin fixed, paraffin-embedded biopsies are however needed to confirm the clinical value of the DNA-ploidy status as a biomarker in BE.

2.5. Inflammation associated markers

Due to gastroesophageal reflux, injured epithelial cells will secrete inflammatory mediators such as cytokines and chemokines, leading to the attraction of inflammatory cells. These inflammatory cells produce reactive oxygen species, that may cause DNA damage and in this way induce tumor promoting mutations [6]. Cyclo-oxygenase-2 (COX-2) is the best known inflammatory enzyme in relation to neoplastic progression in BE.

2.5.1. COX-2

COX-2 is an enzyme, which is induced by inflammatory stimuli and cytokines, and catalyses the synthesis of prostaglandins from arachidonic acid. These prostaglandins stimulate cancer cell proliferation, inhibit apoptosis, and enhance cancer-induced angiogenesis and invasiveness [15].

In most studies, a high expression level of COX-2 in HGD and EAC has been demonstrated [48,49,60,83]. There is however conflicting evidence as to whether COX-2 is involved in early development of EAC, since levels of COX-2 vary considerably in BE patients with ND or LGD [55]. Some studies have shown no differences between ND and LGD [49,60], whereas others reported a progressive increase in COX-2 expression along the metaplasia–dysplasia–adenocarcinoma sequence [48,83]. Cheong et al. reported an increased COX-2 expression in HGD (60 arbitrary units (A.U.; value of density)) compared to non-dysplastic BE (39 A.U.), however COX-2 expression in EAC (46 A.U.) was decreased compared to HGD and not significantly different from ND [15]. In a study by Murray et al., the combination of COX-2 expression and p53 expression was associated with an increased risk of neoplastic progression (OR 27.3), although this combination was only present in 15% of patients who developed EAC [61].

Different techniques have been used to evaluate COX-2 expression, such as immunohistochemistry [15,49,60], Western-blotting [60] or reverse transcriptase/real time polymerase chain reaction [48,49,82]. Inconsistent results have been reported for all three techniques. Therefore, COX-2 is yet not reliable enough to be used as biomarker for determining neoplastic risk in BE.

3. Conclusion

It is generally accepted that the development of EAC in BE is a gradual process in which the disruption of biological processes at the cellular level is accumulating in the cascade from non-dysplastic BE, through LGD and HGD, and finally EAC [12,32,33,35]. At present, histological assessment of the degree of dysplasia is the gold standard for determining risk of neoplastic progression in BE. This histological result determines the frequency of endoscopic surveillance, according to the guidelines of the American College of Gastroenterology [77]. Several studies have evaluated various biomarkers that may assist in determining the risk of progression from BE to EAC. In Table 1 the pros and cons of the biomarkers discussed in this review are summarized. Although some biomarkers, such as DNA ploidy, p53 and Ki67, seem promising candidate markers, either as an additional marker to or even as substitute for histology, contradictory findings have been reported. Moreover, there is a paucity of large prospective follow-up studies. For these reasons, biomarkers are not ready yet for use in daily clinical practice. One of the reasons that only a few large follow-up studies have been performed is the clinical observation that, although increased, the incidence of EAC in the whole group is still relatively low with a frequency of 1 in 200 BE patients per year [16,20,31]. Consequently, it is difficult to perform adequately powered prospective studies investigating the predictive value of various biomarkers, unless performed in a multicenter setting.

Furthermore, it seems likely that panels of biomarkers are more helpful in predicting cancer risk in BE compared to a single biomarker. For instance, Galipeau et al. recently showed that the combined used of the biomarkers 17p13 LOH (p53), 9p21 LOH (p16), and DNA ploidy improved the detection of the subgroup of BE patients with an increased risk of progression to EAC, compared to using only a single biomarker [24]. Therefore, future studies aiming on risk stratification in BE should be performed in a multicenter setting in order to investigate large cohorts of BE patients that could withstand rigorous statistical analysis, and these studies should investigate the use of panels of biomarkers. We are convinced that in the future biomark-

Table 1

Summary of the presently available biomarkers in Barrett esophagus and their pros and cons as biomarker for predicting an increased risk of cancer development in BE

Biomarker	Type of change	Pros	Cons
PCNA	Increased expression with proliferation	Easy to perform	Also stains resting cells
Ki67	Increased expression with proliferation	Easy, stains only proliferating cells	No large-scale longitudinal studies
p53 IHC	Abnormal protein expression	Easy to perform, cheap	Stains also wild-type p53, false negative results by truncated protein
LOH	Frequent LOH at 17p13	Positive large prospective study	Limited availability
p16	LOH at 9p21, early lesion	Common event	Limited availability, no large-scale studies
Cyclin D1	Increased expression	Easy to perform	Contradictory findings, no large-scale studies
β-catenin	Increased nuclear expression, decreased membranous expression		No large-scale studies
DNA ploidy	Aneuploidy with progression	Positive prospective studies performed on fresh tissue	More prospective studies needed on paraffin-embedded tissue
COX-2	Increased expression		Contradictory findings

IHC, immunohistochemistry; LOH, loss of heterozygosity; PCNA, proliferating cell nuclear antigen; COX-2, cyclo-oxygenase-2.

ers will allow a more accurate prediction of the risk of neoplastic progression. In the future, further technological developments will allow that these biomarkers can probably be determined in a (semi-)automated setup, eliminating observer bias and thus adding to, or even replacing, the 'classic' and rather labour-intensive histopathologic evaluation.

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