Mutational load may predict risk of progression in patients with Barrett's oesophagus and indefinite for dysplasia: A pilot study

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Mutational load may predict risk of progression in patients with Barrett’s oesophagus and indefinite for dysplasia: a pilot study

Arvind J Trindade, Matthew J McKinley, Mohammad Alshelleh, Gabriel Levi, Molly Stewart, Kathy J Quinn, Rebecca M Thomas

ABSTRACT

Background and aims Mutational load (ML) has been shown to help risk-stratify those that may progress from non-dysplastic Barrett’s oesophagus (BE) to dysplastic disease. Management of patients with BE and indefinite for dysplasia (BE-IND) is challenging and risk stratification tools are lacking. The aim of this pilot study is to evaluate the utility of ML for risk stratification in patients with BE-IND.

Methods This is a single-centre, retrospective pilot study evaluating ML quantification in patients with BE-IND. Histology at follow-up endoscopy at least 1 year after the baseline endoscopy was used to determine if a patient progressed to low or high dysplasia. The ML levels were then compared among patients who progressed to dysplasia versus those who did not.

Results Thirty-five patients who met the inclusion criteria were identified, and seven met the exclusion criteria. Twenty-eight patients were analysed, of whom eight progressed to low-grade dysplasia (6) and high-grade dysplasia (2). Seven of these eight patients had some level of genomic instability detected in their IND biopsy (ML ≥0.5). Ten of the 20 (50%) who did not progress had no ML level. At an ML cut-off above 1.5, the risk of progression to high-grade dysplasia was 33% vs 0% (p=0.005), with a sensitivity of 100% and a specificity of 85%.

Conclusion These results indicate that ML may be able to risk-stratify progression to high-grade dysplasia in BE-IND. Larger studies are needed to confirm these findings.

INTRODUCTION

Barrett’s oesophagus (BE) is defined as a change from normal oesophageal squamous epithelium to metaplastic columnar epithelium with goblet cells, usually in association with gastro-oesophageal reflux disease. It is a major risk factor for oesophageal adenocarcinoma. Despite recommendations for screening and surveillance in Barrett’s, the incidence of oesophageal adenocarcinoma continues to rise. It is estimated that 5.6% of the adult population have BE in the USA. The only currently used biomarker for risk stratification of BE is dysplasia. However finding and appropriately classifying dysplasia can be difficult. Often dysplasia is focal and finding it can be challenging given most sampling techniques sample a minority of the Barrett’s mucosa. Dysplasia in BE has been classified in a three-tier system as follows: indefinite for dysplasia (IND), low-grade dysplasia (LGD), and high-grade dysplasia (HGD). IND is a category where the observed architectural and nuclear abnormalities are less diagnostic than those seen in clear-cut dysplasia, or when there are significant architectural/cytological atypical features but also significant concomitant inflammation such that reactive atypia cannot be excluded. In clinical practice LGD and IND are often treated the same way in regard to surveillance. However, with the recent shift in the guidelines to ablation of LGD due to a randomised control
trial showing benefit in regard to lack of progression to neoplasia, it is a bit unclear clinically how to manage IND patients.\(^6,7\) In addition, a recent multicentre study has shown that patients diagnosed with IND behave similarly to LGD in regard to biological behaviour.\(^5\) Triaging IND patients according to risk of future progression would help to limit unnecessary repeat endoscopies in patients at low risk and justify closer observation in patients at higher risk, perhaps even supporting early means of cancer prevention such as ablation.

Thus, other biomarkers may be helpful in risk stratification in BE-IND. Currently, dysplasia is the only biomarker used to risk-stratify BE. Despite its widespread use, it is unclear whether surveillance endoscopy to detect this biomarker is useful.\(^6\)\(^-\)\(^11\) As a result research for other biomarkers, particularly molecular biomarkers, to help risk-stratify BE is under way.\(^12\)\(^-\)\(^15\) One potential biomarker that has been identified is mutational load (ML) as a measure of genetic aberration and instability.\(^16\) ML provides a measure of cumulative genomic instability at 10 key genomic loci in patients with BE by assessing DNA damage in proximity to tumour suppressor genes associated with progression to HGD and EA. BE tissue with a higher degree of genetic aberrations, specifically loss of heterozygosity (LOH) of tumour suppressor genes, progresses to more advanced disease.\(^17\)

ML assessment can be determined using a commercially available test (BarreGen, Interpace Diagnostics, Pittsburgh, Pennsylvania, USA). The test quantifies the degree of cumulative genetic derangement of 10 genetic loci of tumour suppressor genes, specifically assessing the presence of LOH mutations and new alleles consistent with microsatellite instability (MSI). The following genetic loci are tested, with their tumour suppressor genes in parentheses: 1p (CMM1, L-myc), 3p (VHL, H6G1), 5q (MCC, APC), 9p (CDKN2A), 10q (PTEN, MXII), 17p (TP53), 17q (RNF43, NME1), 18q (SMAD4, DCC), 21q (TFF1, PSEN2) and 22q (NF2).\(^16\)\(^-\)\(^18\) All LOH mutations are assigned a numerical value based on the degree of derangement. ML consists of a statistically derived weighted scoring system from 0 to 10, with 0 representing the lowest level of genomic instability and 10 representing the highest level of genomic instability.\(^16\)\(^-\)\(^19\)

A recent case–control study evaluated the utility of ML in predicting progression to neoplasia (HGD or intramucosal cancer) based on samples of only BE with LGD or non-dysplastic BE at baseline.\(^18\) Cases that progressed to neoplasia on follow-up (23 patients) were compared with controls (46 patients who did not progress to neoplasia). The mean ML was higher in cases that progressed than controls (2.21 vs 0.42, \(p<0.001\)). The study concluded that ML in preprogression tissue can predict progression to neoplasia in BE and thus may serve as a useful biomarker in surveillance of BE.

ML is proportional to the degree of dysplasia, and thus may serve as an adjunctive test in patients with equivocal histology. A retrospective study looked at 271 patients with varying degrees of dysplasia (IND, LGD and HGD).\(^16\) The authors found that the ML correlated to the grade of dysplasia (1.1 vs 2.2 vs 3.3, respectively; correlation coefficient=0.60, \(p<0.0001\)). The authors concluded that ML may be a useful adjunct to histological evaluation. Another retrospective study examined 877 microdissected targets from BE biopsies. Increasing ML correlated to increasing severe histology in regard to grade of dysplasia (correlation coefficient=0.68, \(p<0.0001\)).\(^19\)

The aim of this study is to determine if ML can help risk-stratify patients with BE-IND.

**METHODS**

This is a retrospective review of patients diagnosed with BE-IND at North Shore University Hospital from 2013 to 2017. A prospectively maintained database was searched for consecutive patients. The inclusion criteria included (1) diagnosis of BE-IND on an endoscopic exam without a concurrent diagnosis of true dysplasia elsewhere in the oesophagus; (2) underwent endoscopic surveillance for IND with Seattle protocol biopsies;\(^20\) (3) underwent ML testing for risk stratification on the index endoscopy biopsies showing IND (preprogression tissue); and (4) had adequate follow-up of at least 1 year if no dysplasia was detected on subsequent exams. Exclusion criteria included the following: (1) underwent endoscopic ablation; (2) lack of 1-year follow-up for patients who did not have dysplasia on a follow-up exam; (3) history of LGD or HGD; (4) dysplasia developing within 1 year of the initial endoscopy (as the dysplasia was likely present on the index exam); (5) presence of oesophagitis on histology or endoscopy; and (6) patient not on a medical antacid regimen.

ML testing was performed on baseline IND biopsy tissue blinded to the future progression status of patients (BarreGen, Interpace Diagnostics). All cases in this series were re-examined by the pathologist and only targets that contained the IND were used. ML was measured using the formalin-fixed, paraffin-embedded (FFPE) tissue from biopsies taken at the time of baseline endoscopy. H&E-stained FFPE slides were microscopically examined by pathologists to identify representative areas of IND histology. H&E-stained slides were used to guide microdissection of recut, unstained, 4 μm thick, FFPE slides. Slides were microdissected for the maximum number of histological targets with IND available for each patient. Microdissection was performed manually, targeting areas in which epithelial cells constituted 90% or more of the total cells removed. By microscopic estimation, no more than 10% of microdissected cells were stromal or inflammatory cells. Accuracy of all microdissections was carefully reviewed by two pathologists.

Selected areas for microdissection contained mainly epithelial cells. DNA from the microdissected targets was then prepared. PCR and quantitative capillary electrophoresis of DNA were used to detect the presence of LOH and new alleles consistent with MSI of the selected DNA markers for the previously discussed 10 genetic loci.
of tumour suppressor genes. For each microdissected tissue, it was determined whether each LOH mutation is of low (50%–75% of the DNA contained LOH) or high (>75% of the DNA contains LOH) mutations) clonality. The sum of the clonality of each genetic loci is the ML.19

Two gastrointestinal pathologists (GL and RMT), with extensive experience in a high-volume Barrett’s tertiary care referral centre, reviewed all initial IND histology and follow-up histology. For the purposes of this study, the histology was rereviewed to ensure IND was an accurate diagnosis prior to preparation for ML analysis. This rereview also served to help locate the area on the tissue where ML would be measured. It should be noted that in our institution, the current clinical practice is that any diagnosis of dysplasia including IND is reviewed at the gastrointestinal pathology consensus meeting, where three to five gastrointestinal pathologists are present.

Patient characteristics were abstracted from the medical chart. Data analysis was separated into two groups. The first group was subjects with BE and IND on the index endoscopy who developed dysplasia (LGD, HGD or intramucosal cancer) on subsequent follow-up at least 1 year after the index pathology of IND. The second group was subjects with BE and IND on the index endoscopy who did not develop dysplasia on subsequent follow-up at least 1 year after the index pathology of IND. These patients had non-dysplastic histology or continued IND histology on follow-up exams. The two groups were compared to determine if there is an ML cut-off that can predict progression to dysplasia or neoplasia (HGD/ intramucosal cancer) in the IND cohort within 1 year of the index endoscopy.

χ² and Fisher’s exact tests were used to compare categorical variables, and the Student’s t-test was used for continuous variables. All analyses were conducted using SAS V.9.4.

**RESULTS**

Thirty-five patients who met the inclusion criteria were identified, and seven who met the exclusion criteria were excluded (three with oesophagitis, three without 1-year follow-up and one not on antacid medication). The study analysed 28 consecutive IND patients at baseline biopsy, 61% were male with a mean age of 64 years (table 1). All IND pathology was confirmed by the pathologists in this study without disagreement. All patients had 1-year follow-up endoscopy, with corresponding follow-up biopsy indicating no progression or indicating progression to LGD or HGD, with 22 patients (79%) having 2 years and 15 patients (54%) having 3 years of endoscopic surveillance follow-up. Of all IND patients, six eventually progressed to LGD (21%) and two to HGD (7%). The baseline mean BE segment length was similar in IND patients who later progressed to LGD (4.0 cm) versus those who did not (3.7 cm), but significantly longer in patients who progressed to HGD (7.5 cm). Of all patients with baseline IND, 29% (8/28) progressed to LGD or HGD, while only 7% (2/28) progressed to HGD.

For analysis, ML scores in IND biopsies were grouped into numerical categories shown in tables 2 and 3. Many IND patients who did not progress to LGD or HGD lacked all detectable genomic instability (10/20) (ML=0; table 2). By contrast 88% (7/8) of patients who progressed to LGD or HGD had at least some level of genomic instability detected in their IND biopsy (ML ≥0.5). The sensitivity and specificity for identifying patients who would later progress to LGD or HGD at this ML threshold were 88% and 50%, respectively (table 2). Using this ML threshold for genomic instability (ML ≥0.5) separated patients who had an initial 29% risk of progression to LGD or HGD at baseline into two, more refined risk categories: (1) those at lower risk of progression to LGD or HGD (9% risk of progression) and (2) those at higher risk of progression to LGD or HGD (41% risk of progression) (9% vs 41%, p=0.07). Higher levels of genomic instability provided higher specificity for predicting which IND patients would progress but at the expense of lower sensitivity for progression.

In contrast, patients who progressed to HGD had comparably higher levels of genomic instability at baseline IND biopsy (ML ≥1.5; table 3). The sensitivity and specificity for identifying patients who would later progress to HGD at this ML threshold were 100% and 85%, respectively. Using this ML threshold for genomic

![Table 1](image1.png)

Table 1 Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>IND progressed to HGD n=2</th>
<th>IND progressed to LGD n=6</th>
<th>No IND progression n=20</th>
<th>All IND n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean</td>
<td>57</td>
<td>61.17</td>
<td>65.2</td>
<td>63.75</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>2/2 (100%)</td>
<td>5/6 (83.33%)</td>
<td>10/20 (50%)</td>
<td>17/28 (60.71%)</td>
</tr>
<tr>
<td>Hiatal hernia</td>
<td>1/2 (50%)</td>
<td>1/6 (16.67%)</td>
<td>7/20 (35%)</td>
<td>9/28 (32.14%)</td>
</tr>
<tr>
<td>Length mean (cm)</td>
<td>7.5</td>
<td>4</td>
<td>3.68</td>
<td>4.04</td>
</tr>
<tr>
<td>Patients had 1-year follow-up exam</td>
<td>2/2 (100%)</td>
<td>6/6 (100%)</td>
<td>20/20 (100%)</td>
<td>28/28 (100%)</td>
</tr>
<tr>
<td>2-year follow-up</td>
<td>2/2 (100%)</td>
<td>4/6 (66.67%)</td>
<td>16/20 (80%)</td>
<td>22/28 (78.57%)</td>
</tr>
<tr>
<td>3-year follow-up</td>
<td>2/2 (100%)</td>
<td>2/6 (33.33%)</td>
<td>11/20 (55%)</td>
<td>15/28 (53.57%)</td>
</tr>
</tbody>
</table>

HGD, high-grade dysplasia; IND, indefinite for dysplasia; LGD, low-grade dysplasia.
instability (ML \( \geq 1.5 \)) separated patients who had an initial 7% risk of progression to HGD at baseline into two, more refined risk categories: (1) those at lower risk of progression to HGD (0% rate of progression) and (2) those at higher risk of progression to HGD (33% rate of progression) (0% vs 33%, \( p=0.005 \)). Again, higher levels of genomic instability provided higher specificity for predicting which IND patients would progress to HGD.

### DISCUSSION

In this study we show that ML can help risk-stratify patients who may progress to dysplasia in patients with BE with IND. In this study 29% progressed to dysplasia (LGD and HGD) and 7% progressed to HGD. This is in line with other single-centre series from tertiary care centre. Previous studies have shown an annual progression rate of 12.9%–25% depending on the series.\(^21\)\(^-\)\(^23\) Seven out of the eight patients who progressed to dysplasia in our study had some level of genomic instability. ML had been shown previously to help risk-stratify patients with non-dysplastic BE or with LGD in regard to progression to HGD.\(^16\) As discussed earlier, these IND patients can be difficult to manage and require more intense surveillance. Thus further risk stratification can be helpful in this subgroup of patients.

This study is novel in that it is the first to examine if ML can be a predictor of progression to true dysplasia in patients with BE and IND. Previous studies have shown ML to be a predictor for progression in non-dysplastic disease.\(^18\)\(^-\)\(^19\) Other studies have looked at DNA content abnormalities from FFPE tissue, such as aneuploidy measured by flow cytometry, in predicting progression from IND to dysplasia.\(^23\)\(^-\)\(^24\) These studies show that DNA flow cytometry can risk-stratify patients with BE-IND who will progress to dysplasia.

Our results show that patients with an ML above 0.5 should be considered for more frequent surveillance compared with those with no ML, as these patients had a higher risk of progression to low-grade and high-grade dysplasia (41% vs 9%, \( p<0.07 \)). The result was not significant likely due to the small sample size. On the other hand, an ML above 1.5 statistically predicted progression to HGD versus those with an ML below 1.5 (33% vs 0%, \( p=0.005 \)). Based on this, an ML above 1.5 should be strongly considered for frequent surveillance and perhaps even advanced imaging if no obvious lesions are visualised given the focal nature of dysplasia.

Not surprisingly, our results are more robust for risk stratification in regard to patients who eventually develop HGD compared with those patients who progressed to LGD. This could be related to the inherent issues with the classification of LGD,\(^25\)\(^-\)\(^27\) which include high interobserver variability among expert pathologists. A sensitivity and specificity of 100% and 85% for an ML above 1.5 for progression to HGD in this cohort support its use as a risk stratification tool in BE for progression to HGD.

The strengths of our study include a true IND cohort. Patients’ histology from preprogression tissue was re-reviewed by experts in gastrointestinal pathology. In addition patients were excluded if there were any signs of

### Table 2 ML performance in predicting future progression to LGD or HGD in IND patients at baseline

<table>
<thead>
<tr>
<th>No IND progression to LGD or HGD</th>
<th>IND progressed to LGD or HGD</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>% IND that progressed to LGD or HGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML=0</td>
<td>10</td>
<td>1</td>
<td>NA</td>
<td>9% of IND progressed (lower risk)</td>
</tr>
<tr>
<td>ML=0.5–0.75</td>
<td>3</td>
<td>3</td>
<td>50</td>
<td>41% of IND progressed (higher risk)</td>
</tr>
<tr>
<td>ML=1.0–1.25</td>
<td>4</td>
<td>1</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>ML=1.5–1.75</td>
<td>2</td>
<td>1</td>
<td>85</td>
<td>38</td>
</tr>
<tr>
<td>ML ( \geq 2 )</td>
<td>1</td>
<td>2</td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>8</td>
<td></td>
<td>Overall 29% of IND progressed</td>
</tr>
</tbody>
</table>

HGD, high-grade dysplasia; IND, indefinite for dysplasia; LGD, low-grade dysplasia; ML, mutational load; NA, not available.

### Table 3 ML performance in predicting future progression to HGD in IND patients at baseline

<table>
<thead>
<tr>
<th>No IND progression to HGD</th>
<th>IND progressed to HGD</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>% IND that progressed to HGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML=0</td>
<td>11</td>
<td>0</td>
<td>NA</td>
<td>0% progressed (low risk)</td>
</tr>
<tr>
<td>ML=0.5–0.75</td>
<td>6</td>
<td>0</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>ML=1.0–1.25</td>
<td>5</td>
<td>0</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>ML=1.5–1.75</td>
<td>2</td>
<td>1</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>ML ( \geq 2 )</td>
<td>2</td>
<td>1</td>
<td>92</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>2</td>
<td></td>
<td>Overall 7% of IND progressed</td>
</tr>
</tbody>
</table>

HGD, high-grade dysplasia; IND, indefinite for dysplasia; ML, mutational load; NA, not available.
oesophagitis on pathology or if patients were not optimised on a medical antacid regimen.

Our study does have limitations. This is a single-centre, retrospective study. We decided to make this a single-centre study to control for heterogeneity in pathological classifications. It also afforded us the opportunity to allow our pathologists to review all baseline pathology. In addition the sample size is relatively small at 28 patients. Another limitation is that despite patients undergoing standard-of-care Seattle protocol biopsies, it is possible that LGD or HGD is missed on the initial exam if a dysplastic area was not seen on endoscopy and missed on random biopsies, especially since this cohort consists of patients with long-segment Barrett’s. This is a known phenomenon that can occur in any surveillance programme. Finally the follow-up period that was required for this study was only 1 year (54% of patients had 3-year follow-up). It is possible that patients who had ML levels above 0.5 and did not progress may progress to low-grade or high-grade dysplasia on further follow-up.

Despite the limitations our pilot study shows that ML may be a useful test for risk stratification in BE-IND. It should be noted that we are not advocating ablation based on ML levels. The decision for ablation should be dictated by the presence of dysplasia, as per the guidelines. However we do feel that ML can dictate surveillance intervals and thus help risk-stratify patients. Larger studies in the future on a BE-IND cohort may give more insight into the sensitivity and specificity of the test. In our study we had one patient with a high ML (>2) with over 3 years of follow-up who never developed dysplasia. On the other hand, we had a patient who developed LGD who never had a measurable ML. Given the small cohort, this affects the sensitivity and specificity of the test, per table 2. Larger studies would be able to account for these extremes and give a better estimate of the sensitivity and specificity of ML in predicting progression to dysplasia. Despite the small numbers it seems that the sensitivity and specificity for development of HGD at ML of 1.5–1.75 (100, 85%) hold promise to be a possible indicator of BE-IND to develop to HGD. Larger prospective observational studies are needed to confirm our findings given only two patients in the cohort progressed to HGD.

In summary, IND patients had a low risk of progression to HGD but a significantly higher risk of progression to any type of dysplasia (LGD or HGD). Genomic instability can further refine risk in these patients by dividing them into categories: (1) those at lower risk of progression to dysplasia and (2) those at higher risk of progression to dysplasia compared with the risk initially conferred by their baseline pathology diagnosis of IND alone. Our results are consistent with those of previous studies demonstrating that ML can be a useful biomarker in identifying patients with BE at risk of future progression to EA, allowing for closer surveillance or cancer-preventative treatment in patients at higher risk of progression and avoiding unnecessary interventions in those at lower risk.

Contributors Conception and design: AJT, RMT. Analysis and interpretation of the data: AJT, MJM, MA, GL, MS, KJQ, RMT. Drafting of the article: AJT, MJM, MA, GL, MS, KJQ, RMT. Critical revision of the article for important intellectual content: AJT, MJM, MA, GL, MS, KJQ, RMT. Final approval of the article: AJT, MJM, MA, GL, MS, KJQ, RMT.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Zucker School of Medicine Institutional Review Board. All research was performed in accordance with all relevant guidelines/regulations and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all participants.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Specific requests for data included in this study can be made to Anind.trindade@gmail.com.

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