Journal for ImmunoTherapy of Cancer

Recommendations for standardizing biopsy acquisition and histological assessment of immune checkpoint inhibitor-associated colitis

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To cite: Ma C, Pai RK, Schaeffer DF, *et al*. Recommendations for standardizing biopsy acquisition and histological assessment of immune checkpoint inhibitor-associated colitis. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004560. doi:10.1136/jitc-2022-004560

Accepted 22 February 2022

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ABSTRACT

Immune checkpoint inhibitor-associated colitis (ICIC) affects approximately 15% of cancer patients treated with immunotherapy. Although histological evaluation is potentially valuable for both the diagnosis of ICIC and evaluation of disease activity, use in clinical practice is heterogeneous. We aimed to develop expert recommendations to standardize histological assessment of disease activity in patients with ICIC. Using the modified Research and Development/University of California Los Angeles (RAND/UCLA) appropriateness methodology, an international panel of 11 pathologists rated the appropriateness of 99 statements on a 9-point Likert scale during two rounds of anonymous voting. Results were discussed between rounds using moderated videoconferences. There are currently no disease-specific instruments for assessing histological features of ICIC. The panel considered that colonoscopy with at least three biopsies per segment from a total of at least five segments, including both endoscopically normal and inflamed areas, was appropriate for tissue acquisition. They agreed that biopsies should be oriented such that the long axis of the colonic crypts is visualized and should be stained with hematoxylin and eosin. Histological items that the panel voted were appropriate to evaluate in ICIC included the degree of structural/architectural change, chronic inflammatory infiltrate, lamina propria and intraepithelial neutrophils, crypt abscesses and destruction, erosions/ulcerations, apoptosis, surface intraepithelial lymphocytosis, and subepithelial collagen thickness. The appropriateness of routine immunohistochemistry was uncertain. These expert recommendations will help standardize assessment of histological activity in patients with ICIC. The panel also identified the development and validation of an ICIC-specific histological index as a research priority.

BACKGROUND

Immune checkpoint inhibitors (ICIs) targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1), and programmed cell death ligand 1 (PD-L1) can prolong progression-free survival and reduce mortality for patients with advanced solid organ and hematological malignancies.[1–5] However, a substantial proportion of patients treated with these immunotherapies will develop immune-related adverse events (IrAEs) as a result of autoimmune cytotoxic T-cell responses that can affect almost any organ system.[6 7] After dermatological manifestations, immune checkpoint inhibitor-associated colitis (ICIC) is the one of the most common organ-specific IrAEs, occurring in approximately 5% to 10% of patients, with higher rates reported in patients treated with combination therapy.[8] Current clinical practice guidelines recommend grading the severity of ICIC using the Common Terminology Criteria for Adverse Events (CTCAE).[9] The CTCAE grading is relatively simple to apply, and the framework is aligned with recommendations for withholding ICIs, introducing corticosteroids, and treating with biologics.[6] However, the CTCAE is predominantly based on patient symptoms rather than objective disease parameters.[10 11]

Theoretically, histology could play an important role in establishing the diagnosis of ICIC, differentiating the condition from other gastrointestinal disorders such as irritable bowel syndrome and infectious enteritis, providing prognostic information, and evaluating response after treatment.[12 13] There is substantial heterogeneity in clinical practice as to when, where, and how histological assessments are applied, and several important questions remain unanswered. First, many patients are treated empirically for ICIC with corticosteroids based on the

clinical symptom of diarrhea without baseline endoscopy or histology. This empiric strategy, while having the advantage of simplicity, potentially results in overtreatment and exposure of patients without ICIC to high-dose corticosteroid therapy. Furthermore, patients who respond to this strategy usually do not undergo post-treatment biopsies to assess for histological improvement, which may be relevant to decision making as their management evolves and resuming immunotherapy is considered. Second, there is no consensus on where and how biopsies should be taken or how biopsies should be processed, stained, and evaluated after procurement. Third, a broad range of histological features have been described in patients with ICIC, yet the clinical implications of these findings are unclear, and our understanding of correlations between different immune markers or cell subpopulations and prognosis is limited.

To address these limitations, an expert panel of pathologists underwent a multiple-round Delphi process using a modified Research and Development (RAND)/University of California Los Angeles (UCLA) appropriateness methodology, with the aim of generating recommendations for standardizing histological assessment of disease activity in patients with ICIC.

METHODS

Statement generation and systematic review

The initial list of items considered for survey development included statements relevant to biopsy acquisition, biopsy processing, histological items and/or indices for measuring disease activity, and other general considerations for histological assessment in ICIC. These statements were informed by a systematic literature review conducted by our group.[14] We searched MEDLINE, Embase, the Cochrane CENTRAL Library, and conference proceedings from Digestive Disease Week, the United European Gastroenterology Week, the Society for Immunotherapy of Cancer Annual Meeting, and the American Society of Clinical Oncology National Meeting to identify studies evaluating disease activity in patients with ICIC. Search terms captured ICIs, diarrhea, and enterocolitis. A total of 5767 citations were identified, 4756 unique records were screened, and 64 studies involving 2809 subjects were included. Full results from this systematic review have been previously published.[14] In brief, although 83% of included studies reported histological findings associated with ICIC, no ICIC-specific histology indices were identified. Several instruments originally developed for ulcerative colitis (UC) have been applied to patients with ICIC, including the Geboes score,[15] Robarts Histopathology Index (RHI),[16] and Nancy index.[17] These indices and other histological measures reported in the literature were used to design the original survey for the RAND.

Expert consensus process

Panel recruitment

An international panel of 11 expert pathologists from the USA, Canada, the UK, and Australia were invited to participate. Panelists were selected on the basis of their expertize in ICIC histological assessment, which took precedence over geographical representation. The final panel selection was determined by CM and VJ.

Modified RAND/UCLA appropriateness methods

We used a modified RAND/UCLA appropriateness methodology to determine the appropriateness and face validity of the statements generated from the systematic review and panel feedback.[18] This evidence-based approach has been widely used and includes a modified Delphi panel method with iterative rounds of anonymous voting followed by group discussions to combine the best available evidence with the cumulative experience of the panel. Specifically, a consensus is not forced, which was important in developing recommendations for ICIC given the relative paucity of evidence for this condition. This approach allows for exploration of concepts potentially relevant to both current applications and future research.

In the first introductory panel meeting, items identified in the systematic review were summarized, and panelists were asked to provide feedback on the clarity of statements and any additional relevant items. The revised survey was then circulated online for all panelists to rate the statements anonymously. Each item was rated for appropriateness on a 9-point Likert scale (1=highly inappropriate, 9=highly appropriate) and was subsequently classified as inappropriate, uncertain, or appropriate according to the median panel score and degree of disagreement. Disagreement was defined as at least three panelists rating the statement at each of the extreme ends of the scale (ie, 1–3 and 6–9). Statements were considered inappropriate if the median score was 1 to ≤3.5 without disagreement, appropriate if the median score was ≥6.5 to 9 without disagreement, or uncertain if the median score was >3.5 to <6.5 without disagreement or if any median score had disagreement present. Median ratings for each statement are reported, along with the distribution of ratings expressed as the mean absolute deviation from the median.

After the first round of voting, all results were summarized and shared with the panelists during a moderated videoconference. Areas of disagreement or uncertainty were reviewed in detail, and panelists were given an opportunity to present arguments in support of or against each statement. The survey was revised according to panel feedback to improve clarity and then recirculated for a second round of anonymous online voting. Final statement appropriateness from the second round of voting was defined as previously described. The discussion highlighted areas relevant to both clinical care and those areas that will benefit from future research.

RESULTS

Overall rating of statements

The first-round survey consisted of 99 statements. Overall, 47 (47%) statements were considered appropriate, 44

(44%) uncertain, and 8 (8%) inappropriate. After a moderated videoconference to review and discuss the results of the first-round survey, an amended final survey was distributed. No items were added after round 1, although there were minor edits to the survey to ensure clarity. In the second-round survey also comprizing 99 statements, 51 (52%) statements were voted as appropriate and 48 (48%) statements were uncertain, with only one of the uncertain statements having disagreement.

General considerations and biopsy acquisition

Ratings for statements relevant to general considerations and biopsy acquisition for assessing histopathology in ICIC are summarized in table 1. Histological evaluation was considered an important measure of disease activity in ICIC and helpful in assessing response to therapy, recognizing that therapeutic response is currently assessed primarily by symptoms alone and that additional research is required to understand the prognostic implications of histological response. Although histological findings may be patchy, a full colonoscopy was considered appropriate to evaluate histological activity in all colonic segments, whereas the appropriateness of sigmoidoscopy alone for assessment of the left colon was uncertain owing to the variable pattern of disease distribution in ICIC. Irrespective of the procedure performed, a uniform biopsy strategy guided by the endoscopic appearance was voted as appropriate for optimally measuring histological activity. Panelists were uncertain whether all biopsies should be taken from the ulcer edge, given that some infectious etiologies are better assessed with biopsies from the ulcer base. Ultimately, taking the biopsy from the most macroscopically abnormal area was voted as appropriate. The use of standard forceps to obtain segmental biopsies, ideally taken at baseline before initiation of therapy, was considered appropriate, as was the acquisition of at least three biopsy samples per segment including samples from endoscopically normal areas. The panel voted that histological assessment of disease activity in ICIC could be challenging to reliably assess in patients with a history of inflammatory bowel disease (IBD), although panelists disagreed on whether the same challenge applies for patients with a history of microscopic colitis. Additional research is required to delineate these populations based on histopathology findings. Finally, the appropriateness of repeating biopsies after treatment to assess histological response to therapy was supported, but the timing of a follow-up endoscopy was uncertain, and additional research is required to understand the dynamics of histological response to therapy. Future studies are encouraged to evaluate different time points for follow-up histology assessment to better characterize the rapidity of histological response.

Biopsy processing

Ratings for statements on biopsy processing are summarized in table 2. As is the standard in clinical care, the need for proper orientation of biopsies in the tissue block, such that the long axis of the colonic crypts is visualized in the tissue section, to accurately score disease activity in ICIC was voted as appropriate. Hematoxylin and eosin (H&E) staining for measuring disease activity was voted as appropriate, whereas immunohistochemistry (IHC) was voted as appropriate for excluding infection with *Cytomegalovirus* (CMV) if suspected on H&E-stained sections. Panelists had a substantial discussion around the potential role of IHC. The appropriateness of routine IHC to quantify various cell types for measuring histological disease activity in ICIC was uncertain, although this area was identified as a research priority. The panel considered the appropriateness of several IHC markers, including CD3, CD4, CD8, Foxp3, CD20, CD68, PD-1, PD-L1, and myeloperoxidase, but all corresponding statements were voted as uncertain.

Histological disease activity in ICIC

Ratings for statements on histological assessment of disease activity in ICIC are summarized in table 3. Overall, it was felt that substantial future research efforts are required to delineate most of the statements considered in this section. The appropriateness of existing histology instruments developed for the assessment of UC, including the Geboes score, the RHI, and the Nancy index, was considered uncertain in the context of ICIC. Therefore, the panel voted that proper development of a novel instrument specific for the evaluation of histopathological activity in ICIC was appropriate. Histology items that were considered appropriate included the degree of structural/architectural change, chronic inflammatory infiltrate, lamina propria neutrophils, epithelial neutrophils, crypt abscesses, crypt destruction, and erosions/ulcerations. The appropriateness of assessing basal plasmacytosis and lamina propria eosinophils was uncertain. Apoptosis was identified as an important measure of histological activity and was voted as appropriate, as was scoring this measure as the number of apoptotic bodies in 10 consecutive crypts. Additionally, withered crypts with apoptosis and/or necrotic debris was voted as an appropriate measure to score.

Surface intraepithelial lymphocytosis and scoring this measure as 0 to 4, 5 to 20, and >20 per 100 colonocytes were considered appropriate for the histological assessment of ICIC. Different definitions of increased intraepithelial lymphocytes were considered, with a threshold of >20 intraepithelial lymphocytes within 100 surface epithelial cells voted as appropriate. The appropriateness of lower (>10 per 100) and higher (>25 per 100) cut-offs was uncertain. Subepithelial collagen scored as normal, patchy thickening, or diffuse thickening was also voted as appropriate, recognizing that this feature may be difficult to differentiate from bona fide collagenous colitis or postulceration scarring.

Panelists were uncertain of the appropriateness of several histological measures of disease activity in ICIC, including the differentiation of surface intraepithelial lymphocytosis from deep crypt lymphocytosis,

Table 1 General considerations and biopsy acquisition for histopathology in immune checkpoint inhibitor-associated colitis

Statement	Median rating (mean absolute deviation from the median)	Clinical care (CC) vs research context (RC)
Histological measurements are important to assess disease activity in ICIC	9 (0.36)	CC
Histological measurements are important to determine therapeutic efficacy after medical treatment of ICIC	8 (1.00)	CC +RC
A full colonoscopy is necessary for evaluation of ICIC histological disease activity in all colonic segments	8 (0.91)	CC
A full ileocolonoscopy is necessary for evaluation of ICIC histological disease activity	6 (1.27)	CC +RC
A sigmoidoscopy is sufficient for evaluation of ICIC histological disease activity in the left colon	5 (1.18)	CC +RC
A uniform biopsy strategy is needed to optimally measure histological disease activity in ICIC	8 (0.73)	CC
The endoscopic appearance of the mucosa should dictate where biopsies are taken from:		
If an ulcer is present, all biopsies should be taken from the edge of the ulcer	6 (1.18)	CC +RC
If ulcers are not seen, but there are macroscopically abnormal areas, biopsies should be taken from the most abnormal area	7 (0.73)	CC
If the endoscopic appearance of the mucosa is normal, biopsies should be taken from random areas	8 (0.91)	CC
Biopsies should be taken from:		
The worst affected area in each of five colonic segments (rectum, sigmoid, descending, transverse, and ascending colon) and the ileum if colonoscopy is performed	7 (0.73)	CC +RC
The worst area in each of three colonic segments (rectum, sigmoid, and descending) if sigmoidoscopy is performed	7 (0.64)	CC +RC
The worst affected area 0–25 cm from the anal verge in order to include the rectum	7 (1.09)	CC +RC
The worst affected area in the rectum	7 (1.27)	CC +RC
The worst affected area in the sigmoid	7 (1.09)	CC +RC
If a certain area was already biopsied, effort should be made to take subsequent biopsies from the same area (even if the mucosa looks improved or normal)	6 (1.27)	CC +RC
If a certain area was already biopsied, subsequent biopsies should be taken from the area of worst endoscopic activity (even if this area is in a different location)	8 (0.91)	CC +RC
The minimum number of biopsies necessary to measure histological disease activity in ICIC is:		
2 biopsies per segment/area biopsied	6 (0.91)	CC
3 biopsies per segment/area biopsied	7 (1.18)	CC
4 biopsies per segment/area biopsied	5 (1.45)	CC
Biopsies should be procured before initiation of ICIC therapy	8 (0.91)	CC +RC
Repeat biopsies after treatment are required to determine treatment response	7 (1.00)	CC +RC
If repeat biopsies are done, the timing of these biopsies after initiation of medical therapy should be:		
2 weeks	5 (0.18)	RC
4 weeks	5 (0.09)	RC
8 weeks	5 (0.18)	RC
12 weeks	5 (0.27)	RC
16 weeks	5 (0.27)	RC
20 weeks	5 (0.36)	RC
24 weeks	5 (0.45)	RC
Standard biopsy forceps should be used to obtain biopsies	7 (0.55)	CC
Jumbo biopsy forceps should be used to obtain biopsies	5 (0.82)	RC
It is acceptable to take biopsies using one bite of the mucosa with one pass of a biopsy forceps	5 (1.00)	CC
It is acceptable to take biopsies using two bites of the mucosa with one pass of a biopsy forceps	5 (1.09)	CC

Continued

Table 1 Continued

Statement	Median rating (mean absolute deviation from the median)	Clinical care (CC) vs research context (RC)
It is acceptable to take biopsies using three bites of the mucosa with one pass of a biopsy forceps	5 (0.64)	CC
Histological disease activity in ICIC cannot be reliably assessed in a patient with a history of inflammatory bowel disease	7 (1.45)	CC
Histological disease activity in ICIC cannot be reliably assessed in a patient with a history of microscopic colitis	4 (1.27)	RC

Green indicates statements voted as appropriate, yellow indicates uncertain appropriateness without disagreement and red indicates uncertain appropriateness with disagreement.
ICIC, immune checkpoint inhibitor-associated colitis.

non-cryptolytic granulomas, mucin depletion, and ischemic injury patterns. This uncertainty was based on the median panel ratings rather than disagreement among the panel; however, panelists expressed that existing evidence supporting use of these items in ICIC is currently insufficient in the literature and should be areas of research priority.

DISCUSSION

Based on the expanded use of ICIs, immunotherapy-related inflammatory toxicities such as ICIC are becoming more common. Current management guidelines recommend empiric symptom-directed treatment for patients with mild diarrhea, with endoscopic and histological evaluation being reserved for patients with more severe disease.[13] However, the role of histopathology in the prognosis and management of ICIC is unclear. As such, substantial heterogeneity remains in clinical practice regarding patient selection for endoscopy, biopsy procurement procedures, and the interpretation of histological findings. Here, we employed RAND/UCLA appropriateness methods to develop expert recommendations

Table 2 Biopsy processing for histopathology assessment in immune checkpoint inhibitor-associated colitis

Statement	Median rating (mean absolute-deviation from the median)	Clinical care (CC) vs research context (RC)
Biopsies should be placed directly in 10% formalin with minimal tissue handling	9 (0.36)	CC
Proper orientation of the biopsies in the tissue block is necessary for accurate scoring of histological disease activity in ICIC	9 (0.73)	CC
Biopsies should be oriented such that the long axis of the colonic crypts is visualized in the tissue section	9 (0.36)	CC
H&E-stained sections are sufficient to measure histological disease activity in ICIC	9 (0.64)	CC
Immunohistochemistry should be performed to exclude infection with cytomegalovirus if suspected on H&E- stained sections	7 (1.55)	CC +RC
Immunohistochemistry should be performed to quantify various cell types to measure histological disease activity in ICIC	5 (1.00)	RC
Immunohistochemistry should be performed for the following markers to measure histological disease activity in ICIC:		
CD3	5 (0.82)	RC
CD4	5 (0.82)	RC
CD8	5 (0.91)	RC
Foxp3	5 (0.82)	RC
CD20	5 (0.82)	RC
CD68	5 (0.82)	RC
PD-1	5 (0.82)	RC
PD-L1	5 (0.82)	RC
Myeloperoxidase	5 (0.82)	RC

Green indicates statements voted as appropriate, yellow indicates uncertain appropriateness without disagreement and red indicates uncertain appropriateness with disagreement.
H&E, hematoxylin and eosin; ICIC, immune checkpoint inhibitor-associated colitis; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1.

Table 3 Histological items for disease activity assessment in immune checkpoint inhibitor-associated colitis

Statement	Median rating (mean absolute-deviation from the median)	Clinical care (CC) vs research context (RC)
The Geboes score should be used as an instrument for assessing histological disease activity in ICIC	6 (0.91)	CC+RC
The Robarts Histopathology Index (RHI) as calculated from the Geboes score should be used as an instrument for assessing histological disease activity in ICIC	6 (1.00)	CC+RC
The Nancy index should be used as an instrument for assessing histological disease activity in ICIC	5 (0.82)	CC+RC
A new instrument is needed to assess histological disease activity in ICIC	8 (1.09)	RC
The degree of structural (architectural) change should be used as a measure for assessing histological disease activity in ICIC	7 (1.00)	CC
In ICIC, the degree of structural (architectural) change should be scored according to the Geboes score as:	7 (1.00)	CC+RC
0 No abnormality		
1 Mild abnormality		
2 Mild or moderate diffuse or multifocal abnormalities		
3 Severe diffuse or multifocal abnormalities		
In ICIC, the degree of crypt architectural distortion (loss of parallel crypt architecture, including the finding of crypt branching, variation in spacing, shape, and size of crypts) should be scored as:	6 (1.00)	CC+RC
0 None (normal)		
1 Mild (focal)		
2 Severe (diffuse)		
The degree of chronic inflammatory infiltrate (lymphocytes and/or plasma cells in lamina propria) should be used as a measure for assessing histological disease activity in ICIC	7 (1.36)	CC
In ICIC, the degree of chronic inflammatory infiltrate (lymphocytes and/or plasma cells in lamina propria) should be scored according to the Geboes score as:	7 (0.82)	CC+RC
0 No increase		
1 Mild but unequivocal increase		
2 Moderate increase		
3 Marked increase		
Basal plasmacytosis should be used as a measure for assessing histological disease activity in ICIC	6 (1.18)	CC
In ICIC, basal plasmacytosis should be scored as:		
Absent or present	6 (1.00)	CC+RC
Absent, focal, or diffuse	6 (1.09)	CC+RC
The degree of lamina propria eosinophils should be used as a measure for assessing histological disease activity in ICIC	5 (0.45)	CC+RC
In ICIC, the degree of lamina propria eosinophils should be scored according to the Geboes score as:	6 (0.73)	CC+RC
0 No increase		
1 Mild but unequivocal increase		
2 Moderate increase		
3 Marked increase		
The degree of lamina propria neutrophils should be used as a measure for assessing histological disease activity in ICIC	8 (1.09)	CC
In ICIC, the degree of lamina propria neutrophils should be scored according to the Geboes score as:	8 (0.73)	CC+RC
0 None		
1 Mild but unequivocal increase		
2 Moderate increase		
3 Marked increase		

Continued

Table 3 Continued

Statement	Median rating (mean absolute-deviation from the median)	Clinical care (CC) vs research context (RC)
The degree of neutrophils in epithelium should be used as a measure for assessing histological disease activity in ICIC	8 (0.91)	CC
In ICIC, the degree of neutrophils in epithelium should be scored according to the Geboes score as:	8 (0.64)	CC+RC
0 None		
1 <5% crypts involved		
2 5%–50% crypts involved		
3 >50% crypts involved		
In ICIC, cryptitis (neutrophils within crypt epithelium) should be scored as absent or present	6 (1.27)	CC+RC
Crypt abscesses (neutrophils within crypt lumens) should be used as a measure for assessing histological disease activity in ICIC	7 (0.82)	CC
In ICIC, crypt abscesses (neutrophils within crypt lumens) should be scored as absent or present	7 (0.55)	CC+RC
The degree of crypt destruction should be used as a measure for assessing histological disease activity in ICIC	8 (1.36)	CC
In ICIC, the degree of crypt destruction should be scored according to the Geboes score as:	7 (1.00)	CC+RC
0 None		
1 Probable—local excess of neutrophils in part of crypt		
2 Probable—marked attenuation		
3 Unequivocal crypt destruction		
The degree of erosion or ulceration should be used as a measure for assessing histological disease activity in ICIC	9 (0.64)	CC
In ICIC, the degree of erosion or ulceration should be scored according to the Geboes score as:	7 (0.91)	CC+RC
0 No erosion, ulceration, or granulation tissue		
1 Recovering epithelium and adjacent inflammation		
2 Probable erosion—focally stripped		
3 Unequivocal erosion		
4 Ulcer or granulation tissue		
In ICIC, ulcers should be distinguished from erosions	7 (1.27)	CC
The degree of apoptosis should be used as a measure for assessing histological disease activity in ICIC	9 (1.00)	CC
In ICIC, the degree of apoptosis should be scored as:		
The number of apoptotic bodies in 10 consecutive crypts	7 (1.00)	CC+RC
The percentage of crypts with ≥1 apoptotic body	6 (1.20)	CC+RC
Absent or present	6 (1.64)	CC+RC
In ICIC, apoptosis should be defined as having more than three apoptotic bodies within the epithelium of 10 crypts	6 (1.18)	CC+RC
The degree of withered crypts with apoptosis and/or necrotic debris (different from a Geboes score of 4.2, which is characterized by attenuated epithelium due to a neutrophilic crypt abscess) should be used as a measure for assessing histological disease activity in ICIC	8 (0.91)	CC+RC
In ICIC, the degree of withered crypts with apoptosis and/or necrotic debris (different from a Geboes score of 4.2, which is characterized by attenuated epithelium due to a neutrophilic crypt abscess) should be scored as:	7 (0.64)	CC+RC
0 None		
1 Rare withered crypt due to apoptosis		
2 Frequent withered crypts due to apoptosis, but not confluent		
3 Confluent withered crypts due to apoptosis		
The degree of surface intraepithelial lymphocytosis should be used as a measure for assessing histological disease activity in ICIC	8 (1.27)	CC

Continued

Table 3 Continued

Statement	Median rating (mean absolute-deviation from the median)	Clinical care (CC) vs research context (RC)
In ICIC, the degree of surface intraepithelial lymphocytosis should be scored as:	7 (0.91)	CC+RC
0–4 per 100 colonocytes		
1 Borderline (5–20 per 100 colonocytes)		
2 Increased (>20 per 100 colonocytes)		
3 Increased (>20 per 100 colonocytes, with associated surface epithelial injury) (different from Geboes scores 5.1 and 5.2 by the absence of neutrophilic inflammation)		
In ICIC, increased intraepithelial lymphocytes (IELs) should be scored as absent or present	7 (1.09)	CC+RC
In ICIC, increased IELs should be defined as having:		
>10 IELs within 100 surface epithelial cells	4 (0.91)	CC+RC
>20 IELs within 100 surface epithelial cells	8 (0.55)	CC+RC
>25 IELs within 100 surface epithelial cells	5 (0.82)	CC+RC
Surface intraepithelial lymphocytosis should be distinguished from deep crypt lymphocytosis for assessing histological disease activity in ICIC	6 (1.36)	CC+RC
In ICIC, the degree of deep crypt lymphocytosis should be scored as:	7 (0.91)	CC+RC
0 Absent		
1 Present, focal		
2 Present, diffuse		
The degree of subepithelial collagen should be used as a measure for assessing histological disease activity in ICIC	7 (1.64)	CC
In ICIC, the degree of subepithelial collagen should be scored as:	7 (1.00)	CC+RC
0 Normal		
1 Patchy thickening		
2 Diffuse thickening		
Granulomas (not cryptolytic) should be used as a measure for assessing histological disease activity in ICIC	5 (0.73)	CC+RC
In ICIC, granulomas (not cryptolytic) should be scored as absent or present	7 (0.91)	CC+RC
Mucin depletion (≤1 goblet cell in 8–10 colonocytes) should be used as a measure for assessing histological disease activity in ICIC	6 (0.91)	CC+RC
In ICIC, mucin depletion (≤1 goblet cell in 8–10 colonocytes) should be scored as absent or present	6 (1.09)	CC+RC
An ischemic injury pattern should be used as a measure for assessing histological disease activity in ICIC	6 (1.00)	CC+RC
In ICIC, an ischemic injury pattern should be scored as absent or present	7 (0.91)	CC+RC

Green indicates statements voted as appropriate, yellow indicates uncertain appropriateness without disagreement, and red indicates uncertain appropriateness with disagreement.
ICIC, immune checkpoint inhibitor-associated colitis.

for standardizing the application of histopathology in patients with ICIC and to encourage additional research for addressing existing knowledge gaps.

The panel unanimously identified the importance of histological measurements for assessing disease activity in patients with ICIC. Patient symptoms, particularly stool frequency and abdominal pain, are emphasized in the CTCAE framework but do not correlate with objective disease measures or response to ICIC treatment and cannot differentiate ICIC from other potential concomitant pathologies such as IBD or CMV colitis.[12 19 20] Rather, endoscopic and histological evaluations remain the reference standard for ICIC diagnosis. Furthermore, more severe histological inflammation has been associated with a more aggressive disease course.[11 12 21–23] In a multicenter study of 149 patients with biopsy-proven ICIC, Pai *et al* identified three distinct histological phenotypes (42% of patients with acute colitis, 45% with chronic active colitis, and 13% with a microscopic colitis pattern), recognizing that some patients may have mixed patterns.[19] These phenotypes cannot be elucidated by symptoms alone. Baseline histological activity measured using the RHI was independently associated with adverse ICIC-related outcomes: the risk of biological-refractory colitis, colectomy, or ICIC-related death was increased ninefold for patients with a baseline RHI score ≥24. These findings

raise the question of whether expanded use of endoscopic and histological evaluation to confirm the diagnosis, evaluate disease severity, and obtain prognostic information might be a preferred strategy over empiric use of corticosteroids.

Although panelists agreed that a uniform biopsy protocol would help standardize histological assessment in ICIC, there was debate around whether a full colonoscopy is necessary or if a sigmoidoscopy is sufficient. Wright *et al* previously conducted a systematic review including 61 studies and 226 cases of ICIC, with approximately half of patients undergoing a full colonoscopy.[24] The authors identified that 98% of reported cases could be diagnosed using a sigmoidoscopy alone based on the presence of distal inflammation. However, pan-colonic involvement was the most common pattern, with 86% (108/125) of patients demonstrating right-sided disease. The panel acknowledged that while a sigmoidoscopy is likely sufficient for diagnostic purposes and does not require oral bowel preparation, a full colonoscopy and segmental biopsies may have better diagnostic and prognostic value because of the potential regional variability in inflammation severity.[25 26] The panel recommended that biopsies should be guided by the endoscopic appearance, similar to guidance provided in UC,[27] recognizing that biopsies from both the edge and center of ulcerations are required for characterizing inflammatory changes and detecting viral inclusions, respectively. Taking biopsies from endoscopically normal mucosa was also considered appropriate given that histological features can occur even in the absence of macroscopic ulceration.[11 12 20]

The panel discussed extensively the histological findings of ICIC. Importantly, there is currently no single criterion that confirms the diagnosis, as none of the histological features are pathognomonic, and there are a broad range of pathological findings that can be observed. Rather, analogous to the situation in IBD, histopathology must be interpreted in the appropriate clinical context with exclusion of other potential etiologies, such as infectious diarrhea. Panelists agreed that the existing histology instruments developed to assess disease activity in UC are not 'built-for-purpose' for ICIC, although many histological features overlap between UC and ICIC.[22] Therefore, the appropriateness of using the Geboes score, RHI, and Nancy index in ICIC was uncertain. Nevertheless, many of the histological items (and corresponding scoring criteria) such as lamina propria and epithelial neutrophils and erosions/ulcerations were considered appropriate measures in ICIC.[28] The panel considered that some studies have successfully used UC histology scoring in patients with ICIC. For example, Cheung *et al* scored biopsy specimens from 134 patients with ICIC using the Nancy index.[22] Their results demonstrated that although the CTCAE grade was not correlated with duration of corticosteroids or requirement for infliximab, patients with a Nancy index score of 3 or 4 were significantly more likely than patients with milder histological inflammation to require biological treatment (50% vs 20%, p=0.03).[22] However, while ICIC has a chronic inflammatory pattern similar to IBD, several histological features were considered less common in ICIC, including basal plasmacytosis. Conversely, the panel discussions emphasized the importance of other features more specific to ICIC for diagnosis, such as apoptosis as well as lymphocytic and collagenous patterns similar to microscopic colitis.[10 29]

Several areas of research priority were highlighted by the panel. First, we identified that the development of valid, ICIC-specific histological instruments would be appropriate to advance the field. Potential applications include over-diagnosis in clinical practice, evaluation of disease activity in both clinical practice and drug development, and prognostication to aid clinical management. Multiple steps are needed to achieve these goals. Our study establishes the appropriateness of potential histological index components that have validity and are feasible to measure. Future work in patients with ICIC is required to determine the intra- and inter-rater reliability of these items and their responsiveness to treatments of known efficacy for ICIC. Second, the panel highlighted the potential value of measuring histopathology after treatment to assess response, although this assessment is not routinely performed in clinical care where therapeutic decisions are based primarily on symptomatic improvement. Additional research is needed to determine the prognostic implications of histological response, particularly for directing decisions around retreatment with immunotherapy.[30] Finally, while the panel voted that H&E staining was sufficient for the assessment of histological activity in ICIC, the precise role of IHC requires further elucidation. Lo *et al* compared biopsy specimens from patients with ICIC to those from patients with IBD and showed not only that the expression of CD8, CD4, and PD-1 was uniquely different among these groups but also that the CD8/FoxP3 ratio and CD68 levels were higher in ICIC patients requiring infliximab than in those who responded to corticosteroids.[31] Understanding how certain immune markers and cell subpopulations correlate with histological activity may provide additional insights into the molecular and immunological determinants underlying disease activity in ICIC and may be of particular interest for identifying novel therapeutic 'upstream' targets for future ICIC treatments.[32 33] However, panelists believe that the additional cost and complexity of IHC would be a limiting factor for its routine use in clinical practice.

Given the composition of the expert panel, we restricted the statements to those most relevant to pathologists. However, several other pertinent questions, such as appropriate selection of patients for endoscopy, timing of procedures, and the use of biomarkers to aid in diagnosis and/or monitoring, warrant discussion. Generally, these are clinical decisions that are made by gastroenterologists or oncologists, falling outside the scope of this specific RAND. Most of these decisions are based on clinical assessment and include consideration of symptom severity, patient comorbidities, potential risks

of endoscopy, endoscopy availability, and response to empiric therapy. Recognizing that endoscopy is a limited resource, biomarkers may play a useful adjunctive role in identifying which patients would benefit most from endoscopic and histological evaluation. For example, fecal calprotectin and lactoferrin are stool-based assays that reflect colonic neutrophil activity and have a high sensitivity and specificity for endoscopic inflammation in patients with IBD.[34 35] Similar associations have been suggested in ICIC.[12 36] For example, in a retrospective cohort of 77 patients with ICIC, Zou *et al* reported that mean fecal calprotectin concentrations were significantly higher in patients with endoscopic ulcerations (641 µg/g) or inflammation (438 µg/g) than in patients with normal endoscopy (263 µg/g, p=0.008).[36] Similarly, patients with acute active colitis histologically had the highest fecal calprotectin concentrations, and among responders to therapy, calprotectin concentrations decreased significantly. The sensitivity and specificity of fecal biomarkers for ICIC will vary depending on the cut-off chosen, and additional research is required to determine optimal thresholds for defining response. In clinical practice, patients with complete symptom resolution and very low calprotectin concentrations (ie, <50–80 µg/g) could potentially defer endoscopy, whereas those patients with equivocal biomarker results or atypical presentations may benefit more from endoscopy and histopathology data.

Our study has some relevant strengths. The study involved an international expert panel of pathologists with experience in assessing ICIC-related biopsies in both clinical care and research settings and used a rigorous methodology to produce recommendations for biopsy acquisition, processing, and interpretation. This work is complementary to several large, ongoing multicenter studies that are prospectively collecting samples from patients with IrAEs. Some examples include the IMMUCARE-BASE study (A Clinical and Biological Prospective Database of Patients Treated with Anticancer Immunotherapy and Follow-up of Their Immune-related Adverse Events, NCT03989323), the MIRAE study (Montreal Immune-Related Adverse Events, NCT05139706), and the Alliance for Clinical Trials in Oncology study (NCT04242095). Collectively, these trials will enroll over 3000 patients and provide researchers with a rich dataset to help understand the operating properties of histopathology in patients with ICIC.

We also acknowledge some important limitations. First, the appropriateness of many statements was uncertain given the relative paucity of evidence for systematic histopathology evaluation in patients with ICIC. Many studies in the literature are retrospective or correlational, limiting the conclusions that could be drawn. Second, we recognize that the clinical care of patients with ICIC is multidisciplinary and frequently involves oncologists, gastroenterologists, internists, and pathologists. We chose to include only pathologists in the panel given the unlikelihood that other specialists would have the technical expertize to provide expert recommendations with respect to histopathology, although we recognize that other important clinical decisions fall outside the scope of these recommendations.

In conclusion, histopathology is an important component of disease activity evaluation in patients with ICIC. Through a modified RAND/UCLA appropriateness process, we have generated expert recommendations for standardizing the acquisition of biopsies, processing of tissue, and assessment of histological items for ICIC, and we have identified areas for future research, including the development and validation of an ICIC-specific histopathology instrument.

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Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests CM has received consulting fees from AbbVie, Alimentiv, Amgen, AVIR Pharma, Bristol Myers Squibb, Ferring, Fresenius Kabi, Janssen, McKesson, Mylan, Takeda, Pfizer, Roche; speaker's fees from AbbVie, Amgen, AVIR Pharma Inc, Alimentiv, Ferring, Janssen, Takeda, and Pfizer; research support from Pfizer. RiKP has received consulting fees from AbbVie, Eli Lilly, Allergan, Genentech, and Alimentiv. DFS has received consulting fees from Merck, Diaceutics, Pfizer, Allakos, and Alimentiv; stock ownership in Satisfai Health. JK has received advisory fees from Clovis Oncology, AstraZeneca, and Tesaro. LG is an employee of Alimentiv. SCM is an employee of Alimentiv. JKM is an employee of Alimentiv. RMF receives

consulting fees from Alimentiv and has received fees from Bristol Myers Squibb, Janssen, and Takeda. RK has received consulting fees from Alimentiv. GYL has received consulting fees from Alimentiv. ReKP has received consulting fees from Alimentiv. CR has received consulting fees from Alimentiv. BGF is a scientific advisory board member for AbbVie, Allergan, Amgen, AstraZeneca, Avaxia Biologics Inc., Boehringer-Ingelheim, Bristol-Myers Squibb, Celgene, Elan, Biogen, Ferring, Genentech–Roche, Janssen–Johnson & Johnson, Merck, Millennium, Nestlé, Novo Nordisk, Novartis, Pfizer, Prometheus, Protagonist, Receptos, Salix, Sigmoid Pharma, Takeda, Teva, TiGenix, Tillotts Pharma, and UCB Pharma; consulting fees from AbbVie, Actogenix, Akros, Albireo Pharma, Allergan, Amgen, AstraZeneca, Avaxia Biologics, Avir Pharma, Axcan, Baxter Healthcare, Biogen Idec, Boehringer Ingelheim, Bristol-Myers Squibb, Calypso Biotech, Celgene, Elan–Biogen, EnGene, Ferring, Genentech–Roche, GiCare Pharma, Gilead Sciences, Given Imaging, GlaxoSmithKline, Ironwood, Janssen Biotech–Centocor, Janssen–Johnson & Johnson, Kyowa Hakko Kirin, Eli Lilly, Merck, Mesoblast Pharma, Millennium, Nestlé, Novo Nordisk, Novartis, Pfizer, Prometheus, Protagonist, Receptos Salix, Sanofi, Shire, Sigmoid Pharma, Synergy Pharma, Takeda, Teva, TiGenix, Tillotts Pharma, UCB Pharma, Vertex, VHsquared, Wyeth, Zealand, and Zygenia; lecture fees from AbbVie, Janssen–Johnson & Johnson, Takeda, and UCB Pharma, and grant support from AbbVie, Amgen, AstraZeneca, Bristol-Myers Squibb, Janssen Biotech–Centocor, Janssen–Johnson & Johnson, Pfizer, Receptos, Sanofi, and Takeda, and is the Senior Scientific Officer of Alimentiv Inc. VJ has received has received consulting/advisory board fees from AbbVie, Alimentiv (formerly Robarts Clinical Trials), Arena pharmaceuticals, Asahi Kasei Pharma, Asieris, Bristol Myers Squibb, Celltrion, Eli Lilly, Ferring, Fresenius Kabi, Galapagos, GlaxoSmithKline, Genentech, Gilead, Janssen, Merck, Mylan, Pandion, Pendopharm, Pfizer, Protagonist, Reistone Biopharma, Roche, Sandoz, Second Genome, Takeda, Teva, Topivert; speaker's fees from, Abbvie, Ferring, Galapagos, Janssen Pfizer Shire, Takeda.

Patient consent for publication Not applicable.

Ethics approval This study does not involve human participants.

Provenance and peer review Not commissioned; externally peer reviewed.

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