Histopathologic tumor regression grading in patients with gastric carcinoma submitted to neoadjuvant treatment
Results of a Delphi survey

Tsekrekos, Andrianos; Detlefsen, Sönke; Riddell, Robert; Conner, James; Mastracci, Luca; Sheahan, Kieran; Shetye, Jayant; Lundell, Lars; Vieth, Michael

Published in:
Human Pathology

DOI:
10.1016/j.humpath.2018.08.028

Publication date:
2019

Document version
Accepted manuscript

Document license
CC BY-NC-ND

Citation for published version (APA):

Terms of use
This work is brought to you by the University of Southern Denmark through the SDU Research Portal. Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim.
Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 06. Apr. 2021
Histopathologic tumor regression grading in patients with gastric carcinoma submitted to neoadjuvant treatment: results of a Delphi survey

Andrianos Tsekrekos, Sönke Detlefsen, Robert Riddell, James Conner, Luca Mastracci, Kieran Sheahan, Jayant Shetye, Lars Lundell, Michael Vieth

PII: S0046-8177(18)30354-X
DOI: doi:10.1016/j.humpath.2018.08.028
Reference: YHUPA 4711
To appear in: Human Pathology
Received date: 27 May 2018
Revised date: 16 August 2018
Accepted date: 21 August 2018


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Histopathologic tumour regression grading in patients with gastric carcinoma submitted to neoadjuvant treatment: Results of a Delphi survey

Andrianos Tsekrekos MD, Sönke Detlefsen MD, PhD, Robert Riddell MD, PhD, James Conner MD, PhD, Luca Mastracci MD, PhD, Kieran Sheahan MB, FRCPath, Jayant Shetye MD, PhD, Lars Lundell MD, PhD, Michael Vieth MD, PhD

aDepartment of Upper Abdominal Surgery, Center for Digestive Diseases, Karolinska University Hospital, Hälsövägen 13, 141 57 Huddinge, Stockholm, Sweden
bDivision of Surgery, Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Hälsövägen 13, 141 57 Huddinge, Stockholm, Sweden
cDepartment of Pathology, Odense University Hospital, Winsløwparken 15, 5000 Odense C, Denmark
dDepartment of Pathology and Laboratory Medicine, Mount Sinai Hospital, Joseph and Wolf Lebovic Health Centre, 600 University Avenue, Toronto, ON M5G 1X5, Canada
eDivision of Anatomic Pathology, Department of Surgical Science and Integrated Diagnostics (DISC), University of Genoa, Via Balbi 5, 16126 Genoa, Italy
fDepartment of Pathology, St Vincent's University Hospital & UCD School of Medicine, Elm Park, Dublin 4, Ireland
gDepartment of Pathology, Karolinska University Hospital, Hälsövägen 13, 141 57 Huddinge, Stockholm, Sweden
hInstitute of Pathology, Klinikum Bayreuth, Preuschwitzer Str. 101, 95445 Bayreuth, Germany

Corresponding author:
Department of Upper Abdominal Surgery, Center for Digestive Diseases
Karolinska University Hospital, Hälsövägen 13, 141 57 Huddinge, Stockholm, Sweden
E-mail address: andrianos.tsekrekos@sll.se
Phone: +46 76 20 63 258, +46 8 58 58 0000

Keywords: Gastric cancer; Histopathologic tumour regression grade; Delphi

Running head: Histopathologic tumour regression grading in gastric carcinoma

Conflicts of interest: The authors declare no conflict of interest.

Funding: No financial support was received from funding agencies in the public, commercial, or not-for-profit sectors.

Summary
Studies investigating the histopathologic response of gastric carcinoma to neoadjuvant treatment have used a variety of different tumour regression grading systems. The aim of this Delphi survey was to review the available systems and reach consensus on a potential international standard. An international email-based Delphi survey involving six expert pathologists was undertaken between January and October 2017. A questionnaire consisting of seventy-two items was formed after reviewing the five available systems. Rating of the items was done on a symmetric 4-point Likert-type scale and feedback was provided between rounds. A total of four rounds were required to reach consensus on 97% of the items covering the topics: (1) Specimen processing, (2) Gross examination, (3) Cross-sectioning / method of sampling, (4) Staining, (5) Immunohistochemistry, (6) Assessment of tumour regression in response to neoadjuvant therapy, (7) Tumour regression grading, (8) Assessment of regression of nodal metastases and (9) Role of histological tumour type. Through the outcome of this comprehensive Delphi study, a group of experts is proposing a 4-tiered system for the grading of regression of the primary tumour, combined with a 3-tiered system for lymph node metastases. Grade 1 represents complete response, grade 2 contains <10% residual tumour (subtotal regression), grade 3 10-50% residual tumour (partial regression) and grade 4 >50% residual tumour (minimal/no regression). The addition of “a”, “b” or “c” indicates complete, partial or no response of lymph node metastases. It is recommended to use this grading system irrespective of histologic subtype.

1. Introduction
Despite the fact that the incidence of gastric cancer is steadily declining, it still represents the fifth most common malignancy and the third leading cause of cancer-related death worldwide [1]. In Western countries the prognosis of gastric cancer remains poor; 5-year survival is reported to be in the range of 25% [2], suggesting that non-detectable metastatic disease - either regional or distant - is already present at the time of surgery in a significant proportion of patients intended to have a potentially curative resection. Given that, a variety of avenues have been pursued in attempts to improve survival rates, mainly by offering systemic treatment in the form of perioperative chemotherapy.

The use of neoadjuvant chemotherapy for gastric cancer was first reported by Wilke et al. in the context of advanced, non-resectable tumours. Second-look surgery occasionally revealed a chemotherapy-induced “downstaging”, or even complete disappearance of all macroscopic disease, making resection possible [3]. Following these landmark observations, several studies were conducted examining the value of perioperative chemotherapy given to patients with locally advanced but resectable tumours, and this strategy appeared to offer survival benefit as demonstrated in three phase III randomized controlled trials [4-6]. These findings translated into a wide-spread use of perioperative chemotherapy, which has become the standard option in Europe [7].

In the ongoing search for more effective multimodal therapeutic protocols in advanced gastric cancer, several phase II trials have been initiated, where one issue is which relevant surrogate markers can predict a significant clinical effect; one is the grade of tumour response to neoadjuvant therapy, where three evaluation criteria
are currently available. The Response Evaluation Criteria in Solid Tumours (RECIST) is considered the gold standard [8]. However, RECIST requires the presence of a measurable lesion and, given the fact that resectable gastric carcinoma seldom has measurable lesions, its use is not a valid option for clinical research in the neoadjuvant setting. The Japanese Classification of Gastric Carcinoma advocates a response evaluation criterion involving barium X-ray or endoscopic examination for the assessment of such tumours without measurable lesions, but until now this method has not been met by a widespread acceptance [9]. Finally, tumour regression grade (TRG) can be evaluated histologically in the resected specimen.

In 2014, Kurokawa and colleagues conducted a correlative study to determine the best surrogate endpoint variable for overall survival in neoadjuvant studies for gastric cancer, stimulated by the finding that in esophageal cancer the histopathologic response rate was accurate and superior to RECIST [10]. The results suggested that histopathologic response is indeed a valid surrogate endpoint variable relevant for survival outcomes and harbours the potential to be used in neoadjuvant trials [11]. Recent studies have provided evidence to support this, confirming the prognostic value of histopathologic tumour regression in gastroesophageal cancer and the correlation of response with a favourable outcome [12, 13]. Such a conclusion has recently been amplified by the results of the FLOT4-AIO trial, which compared two different perioperative chemotherapy regimens in resectable gastric and gastroesophageal junction cancer; the primary endpoint of the phase II part of the trial was the proportion of patients with pathological complete regression (pCR) and the analysis revealed a 10% difference in the rate of pCR
between the groups [14]. Subsequently, this was reflected in the results of the phase III part of the trial, demonstrating a corresponding difference in overall survival [6].

In studies investigating the histopathologic response to neoadjuvant therapy in gastric cancer, several different TRG systems have been used [9, 15-18]. The common feature of these systems is that they are based on the estimated percentage of, either residual viable tumour tissue in relation to the initial tumour bed, or the amount of therapy-induced fibrosis in relation to residual tumour. This diversity of the grading systems encountered in the literature complicates the interpretation and generalizability of research data, making cross-trial comparisons redundant. The aim of this Delphi survey was to comprehensively review these TRG systems with the objective to either reach consensus on which one has the potential to become the international standard, or alternatively launch a novel system for this purpose.

2. Methods

2.1. Identification and selection of participants - panel size

Candidates were invited due to their special interest, scientific and clinical competence in the field of gastrointestinal pathology, whereupon six experts finally agreed to participate. This approach did not allow for true anonymity, as the participants were known to the researchers and partially even to one another. Nevertheless, the respondents’ judgments remained strictly anonymous to the expert panel throughout the survey. Correspondence was email-based and we obtained a response rate of 100% in all rounds.
2.2. Brief description of the Delphi survey process

The Delphi survey was based on a process consisting of a total of four rounds, with interspersed controlled feedback. A search of the relevant literature had identified five different systems for the histopathologic assessment and classification of tumour response in gastric cancer [9, 15-18]. For the first round, we proceeded with the development of an evaluation form for each of the systems, presenting their respective characteristics. We also added the two most commonly used systems for esophageal and GEJ cancers [19, 20], in order for the participants to reflect on their potential application in gastric cancer as well (appendix A). The experts were asked to evaluate the different elements of each individual system and express their opinion on their pros and cons. Additionally, there was ample space for comments and proposals, with the purpose to identify issues to be addressed in subsequent rounds. Based on the collected responses, a questionnaire consisting of 72 statements was formed, covering the topics: (1) Specimen processing, (2) Gross examination, (3) Cross-sectioning / method of sampling, (4) Staining, (5) Immunohistochemistry, (6) Assessment of tumour regression in response to neoadjuvant therapy, (7) Tumour regression grading, (8) Assessment of regression of nodal metastases and (9) Role of histological tumour type (appendix B). In round 2 the participants were asked to give their opinion by rating each of the 72 statements on a symmetric 4-point Likert-type scale ranging from 1 (completely disagree) to 4 (completely agree). Consensus was achieved in 53 of 72 items (74%), whereupon a new questionnaire was constructed for round 3, consisting of the items where consensus had not been reached and incorporating feedback in terms of the results.
of the previous round (appendix C). In this next round, the statements were either slightly rephrased, in order to integrate participants’ proposals, or remained unchanged and the panellists were provided with the other experts’ original remarks instead. In a number of items the choice was restricted to a “yes” or “no” answer. Finally in round 4, one single question was distributed, with the sole objective to consent on the exact form of the TRG system to be recommended (appendix D). After obtaining consensus in 97% of the statements and with a majority of 4 participants advocating the same system, we decided to terminate the survey and summarize the results. A more detailed description of the survey process is provided in appendix E.

2.3. Data analysis, statistical interpretation and definition of consensus

To date, no universally accepted criterion exists when it comes to determine consensus on Delphi surveys and a variety of statistical analysis techniques for interpreting the data are encountered in the literature. We therefore consulted a professional statistician who assisted us in defining consensus as follows: based on the participants’ scoring on the previously described 4-point scale, a median value was calculated and consensus was considered to exist if the median was either ≥ 3.0 (corresponding to completely or partly agreement from the majority) or ≤ 2.0 (corresponding to completely or partly disagreement from the majority). On the contrary, consensus was not reached on the statement in question if the median was between 2.1 and 2.9.
3. Results

Herein we present the results of the Delphi survey and recommendations concerning the assessment and grading of tumour regression in the primary tumour and in nodal metastases. The detailed outcomes of the survey regarding the processing and gross examination of the resection specimen, as well as the cross sectioning/method of sampling, staining and immunohistochemistry are presented in Table 1.

3.1. Assessment of tumour regression in response to neoadjuvant therapy

Microscopic tumour regression should be determined semi-quantitatively (no need to calculate a precise percentage value), based on an estimation of the amount of residual viable neoplastic tissue in relation to the total assessable carcinoma area, including areas with chemotherapy-induced tissue injury (fibrosis or fibro-inflammation).

Viable tumour cells are defined as those cells which are judged to be capable of proliferation. Nevertheless, this is highly subjective and it may sometimes be impossible to conclude with absolute certainty whether a given tumour cell is viable or not. Likewise, differentiating between desmoplastic stroma, which is also neoplastic, and regressive stroma, which is an expression of response to therapy (treatment-related) is also challenging. In assessing the relative amount of residual tumour/regressive changes, not only the cancer cells, but also the desmoplastic stroma in their immediate vicinity should be interpreted as “cancer bed”, unless
obvious regressive fibrosis is seen. In these latter cases the stroma in the immediate vicinity of the cancer cells should be interpreted as a sign of regression. “Dirty” necrosis, i.e. necrosis consisting of nuclear debris, admixed and bordered by viable tumour cells, should not be interpreted as a sign of response. Only “infarct-like” ischaemic necrosis, consisting of eosinophilic cytoplasmic remnants but lacking viable tumour cells, should be interpreted as a sign of response.

3.2. Grading of tumour regression

During the initial evaluation of the TRG systems in round 2, only one participant advocated the use of a 5-tiered system, while the remaining five recommended either a 3- or a 4-tiered system. In the third round, the majority of the experts (4 out of 6) supported the use of a 4-tiered system. Subsequently, at the final round, a 4-tiered system based on modified Becker’s grading system [15] was proposed by the majority:

- Grade 1  No residual tumour (complete response)
- Grade 2  <10% residual tumour (subtotal regression)
- Grade 3  10-50% residual tumour (partial regression)
- Grade 4  >50% residual tumour (minimal/no regression)

Examples of representative histological images are provided in Figure 1 (A-D).

3.3. Assessment of regression of nodal metastases

There was total agreement among the participants that, apart from the conventional ypN stage (i.e. total number of lymph nodes identified, number of lymph nodes with signs of metastatic involvement), the pathologist should also record the presence of
any signs of tumour regression in the involved lymph nodes (i.e. acellular mucin lakes, fibrosis or aggregates of foamy macrophages). That is, assessment of regression is needed for lymph node metastases as well, but in contrast to the primary tumour, only qualitative assessment and description suffice and can be simplified using 3 categories, summarizing the findings in all the examined lymph nodes as follows:

- Grade a Complete response (only fibrosis or mucin lakes without viable cells)
- Grade b Partial response (viable neoplastic cells together with regressive changes)
- Grade c No signs of tumour response

Examples of representative histological images are provided in Figure 2 (A-C).

3.4. The role of histological tumour type

Consensus was reached on the question whether the selection of procedures and the method of evaluation should depend on the histological type of the tumour. A unanimous recommendation is that these should be the same irrespective of histological type (Laurén, WHO) or the presence of any possibly high-risk subtypes [21-23].

4. Discussion

The Delphi method is frequently used in clinical research areas that are dominated by significant diversities in opinions, substantial amount of information in the available literature which is mostly of limited scientific grade, and where a
corresponding process is intended to form the platform for the launch of a subsequent research plan aiming to offer data of higher scientific grade. It has to be recognized that there are methodological issues in general confined to the application of the Delphi method. Among others, there appears to be little agreement regarding the size of the panel required, how to identify and select appropriate participants, or how to manage the data that the process generates [24-26]. Nevertheless, it is considered the best way to gain the most reliable consensus of opinion of a group of experts.

The aim of this Delphi study was to, taking base from the existing TRG systems in gastric cancer, generate a novel system avoiding the draw backs and limitations of some and adopting the perceived advantages of others. Another objective was to develop and specify the details of a protocol which would secure adequate and standardized histopathologic assessment; from the specimen processing and gross examination, to the method of sampling, the standard staining and possible supplementary use of immunohistochemistry, to the way of estimating the tumour regression in the histological slides. This need for international agreement is emphasized in the latest ESMO Clinical Practice Guidelines on gastric cancer, which state that “There should be national or preferably international guidelines for dissection and reporting” [7]. Mainly, we aimed to reach consensus on the optimal way to stratify TRG in both the primary tumour and the nodal metastases. This methodology differs from alternative-complementary approaches, as represented by a recent critical review of the relevant literature by Langer and Becker [27]. A total of four rounds were required to reach consensus on the vast majority of items (97%), with some areas deserving further discussion.
The issue of whether one should proceed with additional sectioning in cases of presumed complete response in order to confirm this finding was proven to be a matter of debate and one of the few topics where consensus was not reached. Half of the experts advocated that it is necessary to confirm this finding by additional sectioning in the area of interest and, if necessary, the whole stomach should be submitted to histologic examination as to allow for a definitive conclusion. According to others, this is not necessary as long as the recommendations regarding the method of sampling are followed correctly (see Table 1, Cross sectioning / method of sampling).

Immunohistochemistry for broad-spectrum cytokeratins (CK IHC) carries a potential role as an adjunct, if standard stains are inconclusive; otherwise its use should not be uncritical and it can currently not be recommended as part of the standard procedure. This section of the survey, covering various aspects on the use of IHC, was the one that generated most comments and, although all statements reached consensus in the final round, responses were accompanied by a plethora of remarks and in some cases with certain reservations expressed by the participants. One issue was whether CK IHC should be an essential part of the next step in cases of initial failure to detect residual cancer cells. Taking into account the considerable cost of an overuse of IHC, a compromise was proposed as an alternative; i.e. to proceed with IHC on a number of selected sections where epithelial cells are not identifiable, but where the pathologist clearly notes the presence of signs of treatment response (fibrosis, accumulation of macrophages, mucin pools, etc) on the initial review of H&E slides. In order to minimise interobserver variability it may be wise to clearly define in advance in which situations CK IHC should be used, e.g. cases with poorly
cohesive carcinoma and overt inflammation. Hence, in such clearly defined cases, where the response is complete or near-complete, a standard CK IHC protocol on a few representative slides should be in place. Future research has to concentrate on these issues to offer robust and cost-effective guidelines.

The expert panel did not identify a need to evolve a novel system; instead, the 4-tiered grading system proposed by Becker and colleagues, with a minor modification, was favoured by the majority (Table 2). The desirability of developing a 3-tiered system emerged, which makes sense as one of the goals in general is to use as few subdivisions as possible. At the same time, complexity must be balanced with maintaining the classification system’s capacity to discriminate between patient groups with different outcomes. In another study based on data from a large number of patients (n=480), multivariate analysis showed that the Becker grading system using 3 grades instead remained an independent prognostic factor for survival. Despite this finding, the authors suggested retaining the subdivision into grade 1a (complete response) and 1b (subtotal response), in order to preserve the information about the presence of complete tumour regression [28]. Similarly, the Delphi panel reached consensus on a 4-tiered system as participants argued that 1) It is important to maintain a distinction between complete response and almost complete response (<10% of tumour remaining) and 2) Grouping together patients with scarce or rare residual tumour cells, i.e. nearly complete response, with those who have up to almost 50% residual tumour is not justified, as such subdivision would probably mask important clinical outcome differences.

The proposed grading system is different from that advocated by Becker [15], since it incorporates grading of tumour response in the nodal metastases. Although it is
undisputable that the lymph node status offers major prognostic information in gastric cancer, previous studies on histopathologic tumour regression focussed almost exclusively on the primary tumour. Nodal regression per se has rarely been studied and therefore its clinical implication remains unclear but emerges as an important target for future studies. Becker and colleagues, recognizing the relevance of lymph node status, developed a multifactorial histopathological prognostic score, combining the classical ypT and ypN parameters with TRG [29]. Ultimately this system could, based on retrospective data analysis, identify three patient groups with significantly different prognosis. Nevertheless, the authors grouped together patients with complete or subtotal regression (<10% residual tumour); our conclusion is that these should remain two distinct subsets of patients. Additionally, we argue that incorporating TRG in metastatic lymph nodes (as an adjunct to conventional ypN status) has the potential to offer a relevant complementary prognostic tool. In contrast to the primary tumour site, we suggest a qualitative assessment and simplified classification into 3 categories, i.e. a) Complete response, b) Partial response and c) No signs of tumour response (Table 2). Regarding the interobserver and intraobserver variability in the assessment of TRG in general and in lymph nodes in particular, this has to be separately studied within the framework of dedicated prospective protocols. The same applies to the correlation of the proposed system with survival outcomes, as corresponding information is not available in the literature.

Another issue addressed in this survey was whether the assessment protocol should be tailored to the histological subtype (Laurén, WHO). This approach, although
interesting, did not gain the panel’s support, as most gastric carcinomas are mixed type and a tailored procedure would complicate an already difficult task.

There are a number of limitations in this study, as well as methodological issues in general, when applying the Delphi method; some unintentional “leading” by the investigators inevitably occurs because of the selection of the information communicated during the process of feedback. The lack of precise definition on what constitutes consensus is another limitation and it should be emphasized that, even if consensus is achieved, the conclusions represent at best an opinion and should be interpreted as such. Indeed, during the survey the participating experts repeatedly stated that, in a number of issues, there is simply not enough evidence to support a conclusion and that more studies are needed. Finally, gastric cancer is common especially in East Asia, where the assessment of TRG follows the guidelines of the Japanese Classification of Gastric Carcinoma [9]. In order to minimize the complexity of the survey we selected only experts from the Western hemisphere. Obviously, for a widespread dissemination of results like ours it is vital that a corresponding survey is conducted in Asia as well and that the proposed system is subjected to the necessary validation in the respective clinical settings.

5. Conclusions

Standardization of the evaluation and reporting of histopathologic TRG after neoadjuvant treatment of gastric carcinoma is warranted and has to be implemented in routine clinical practice, as well as in research projects. Through the outcome of a comprehensive Delphi survey, an international group of experts
proposes a 4-tiered system for the grading of regression in the primary tumour, combined with a 3-tiered system for the metastatic lymph nodes. In this system, grade 1 contains no residual tumour (complete response), grade 2 <10% residual tumour (subtotal regression), grade 3 10-50% residual tumour (partial regression) and grade 4 >50% residual tumour (minimal/no regression). The addition of “a”, “b” or “c” indicates complete, partial or no response in the metastatic lymph nodes. It is recommended to use this tumour regression grading system irrespective of the histological subtype of gastric carcinoma.

Acknowledgments

Donal Barrett, statistician at the Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet is acknowledged for providing advice on the analysis and interpretation of data.

Dr Balint Melcher, Institute of Pathology, Klinikum Bayreuth, is acknowledged for providing representative histological images.

Appendices

Appendix A – Questionnaire Round 1
Appendix B – Questionnaire Round 2
Appendix C – Example of Questionnaire Round 3
Appendix D – Questionnaire Round 4
Appendix E – Detailed description of the Delphi survey process
References


Charalampakis N, Nogueras Gonzalez GM, Elimova E, et al. The Proportion of Signet Ring Cell Component in Patients with Localized Gastric Adenocarcinoma Correlates with the Degree of Response to Pre-Operative Chemoradiation. Oncology 2016; 90, 239-47.


Fig 1. Representative histological images of the four grades of tumour regression – Primary Tumour (Hematoxylin-eosin staining; 25x magnification): A. Primary tumour bed showing no vital tumour cells after chemotherapy and reactive changes of non-neoplastic mucosa at the upper left hand side of the image – Grade 1; B. G2 adenocarcinoma with less than 10% vital tumour cells and around 10% mucinous component left on mucosal niveau with marked fibrosis underneath and regression for more than 90%. Additionally, there is one lymphatic vessel permeation visible underneath the tumour at the bottom of the image, right lower half – Grade 2; C. G2 adenocarcinoma showing fibrosis after chemotherapy within and around the primary tumour – Grade 3; D. G2 adenocarcinoma showing no regression after chemotherapy – Grade 4.
Fig 2. Representative histological images of the three grades of tumour regression – Metastatic Lymph Nodes (Hematoxylin-eosin staining; 25x magnification): A. G2 adenocarcinoma former lymph node metastasis with fibrosis after chemotherapy with no viable tumour cells – Grade a; B. G2 adenocarcinoma lymph node metastasis showing little fibrosis after chemotherapy between the tumour sheets in a case of regression grade 4 of the primary tumour – Grade b; C. G2 adenocarcinoma showing no regression of a lymph node metastasis in case of no regression of the primary tumour – Grade c (non-responder).
<table>
<thead>
<tr>
<th>Topic</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| **Specimen processing** | - The specimen has to be sent to the pathology department fresh and intact, optimally in a vacuum assisted, temperature controlled system. If such a system is not available, conventional sending procedures should be applied, with cold storage at 4°C.  
- In case of longer distances of transportation, fixation in neutral buffered formalin is mandated. |
| **Gross examination** | - After documentation of any serosal tumour involvement, the stomach should be opened by dividing the wall opposite to the tumour (the opening of the specimen needs to be case dependent).  
- In cases of circumferential tumour growth or no macroscopic residual tumour, the specimen should be opened along the greater curvature.  
- Photographic documentation of the specimen should be done routinely (ideally fresh).  
- It is of value to divide the specimens into three macroscopic groups, as described by Mandard et al:  
  1. Obvious residual tumour with ulcerating, fungating or infiltrative features  
  2. Apparent tumour regression has occurred, a scar is found instead  
  3. Doubtful cases with partial response  
   Depending on the gross aspect of the residual tumour, a different sampling method can be considered (see below, Cross-sectioning/method of sampling).  
- The adipose tissue located underneath the residual neoplasm should not be dissected to search for lymph nodes before pinning but after fixation and tumour sectioning. |
| **Cross sectioning / method of sampling** | - The specimen should be fixed in 10% buffered formaldehyde for 24-48 hours before sampling.  
- In case of “fresh” sampling for bio-banking or when fresh tissue for molecular analysis is needed, tissue should be frozen (ideally in OTC media) until final histologic examination of the specimen is reported, in case it needs to be reevaluated.  
- The fixation period should not exceed 72 hours, since this creates excessive formalin mediated cross linking, leading to potential problems with immunohistochemistry and other molecular techniques.  
- Representative sections should include the region of the tumour, surgical margins and uninvolved portions of the stomach as a reference to assess background mucosal changes/pathology on which the carcinoma arose (adequate sections of antrum, corpus and fundus).  
- Cross-sections should be captured serially at 3-5 mm intervals from:  
  1. The entire macroscopically identifiable tumour  
  2. Areas with abnormalities (i.e. increased consistency or thickness of the wall, ulceration)  
  3. The area of the stomach with scarring, indicating the site of the previous tumour  
- Sampling should be extended to include 2 cm of the adjacent tissue around the tumour. The same sampling strategy should be applied in the situation with no macroscopically identifiable tumour, since subjecting only ulceration/scar tissue for histology may underestimate tumour response.  
- In the case of no gross identifiable tumour bed, correlation with pre-therapy endoscopy, previous histology reports or imaging findings should be considered.  
- In large tumours that macroscopically seem not to have responded to therapy, it may be sufficient to only submit every second slice for histological examination. |
| **Staining** | - Initial assessment by hematoxylin & eosin (H&E) only is sufficient.  
- The use of additional stains depends on what is visible on the H&E.  
- Alcian Blue-PAS can be used to assist in distinguishing signet ring cells (or other more immature subtypes) from histiocytes. However, the use of immunohistochemistry for broad-spectrum cytokeratins is recommended for this purpose, due to its superior specificity. |
|  | - There is no need for the upfront use of IHC in the routine assessment of tumour regression grade, |
**Immunohistochemistry (IHC)**

- but it can be added depending on what is visible on the standard stains.
- The supplementary use of IHC depends more on the degree of tumour response to therapy, rather than its histologic type.
- IHC techniques to determine the expression of broad-spectrum cytokeratin-positive cells carry a potential to improve the identification of viable tumour cells within large areas of fibrosis. This may be useful in cases of “no residual carcinoma” diagnosed on the H&E staining, but also in cases with up to 10% residual tumour. Above all, it may be useful in diffusely infiltrating, poorly cohesive/signet ring carcinoma, where tumour cells can be difficult to recognize (and quantify) on a background of post-chemotherapy inflammation with macrophages and fibrosis.

**Table 1**  Outcomes of the survey and recommendations regarding the specimen work up (specimen processing, gross examination, cross sectioning/method of sampling, staining and immunohistochemistry)
<table>
<thead>
<tr>
<th>Tumour Regression Grade</th>
<th>Criteria</th>
<th>Classification of response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Tumour</strong></td>
<td>% viable tumour</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>No residual tumour</td>
<td>Complete regression</td>
</tr>
<tr>
<td>Grade 2</td>
<td>&lt;10% residual tumour</td>
<td>Subtotal regression</td>
</tr>
<tr>
<td>Grade 3</td>
<td>10-50% residual tumour</td>
<td>Partial regression</td>
</tr>
<tr>
<td>Grade 4</td>
<td>&gt;50% residual tumour</td>
<td>Minimal/No regression</td>
</tr>
<tr>
<td><strong>Metastatic LNs</strong></td>
<td>Microscopic findings</td>
<td></td>
</tr>
<tr>
<td>Grade a</td>
<td>Only fibrosis or mucin lakes without viable cells</td>
<td>Complete regression</td>
</tr>
<tr>
<td>Grade b</td>
<td>Viable neoplastic cells together with regressive changes</td>
<td>Partial regression</td>
</tr>
<tr>
<td>Grade c</td>
<td>No signs of tumour response</td>
<td>No regression</td>
</tr>
</tbody>
</table>
Table 2 Proposed system for the grading of histopathologic tumour regression in gastric adenocarcinoma submitted to neoadjuvant therapy in the Primary Tumour and Metastatic Lymph Nodes (LNs)
Histopathologic tumour regression grading in patients with gastric carcinoma submitted to neoadjuvant treatment: Results of a Delphi survey

Andrianos Tsekrekos MD · Sönke Detlefsen MD PhD · Robert Riddell MD PhD · James Conner MD PhD · Luca Mastracci MD PhD · Kieran Sheahan MB FRCPart · Jayant Shetye MD PhD · Lars Lundell MD PhD · Michael Vieth MD PhD

Corresponding author:
Andrianos Tsekrekos MD
Department of Upper Abdominal Surgery
Center for Digestive Diseases
Karolinska University Hospital, Stockholm, Sweden
E-mail: andrianos.tsekrekos@sll.se

Highlights

- Standardized evaluation of the histopathologic tumour regression grade is warranted
- The proposed grading system is the result of an international Delphi survey
- Regression of the primary tumour is stratified on a 4-tiered system (Grade 1-4)
- “a”, “b” or “c” indicates complete, partial or no response of lymph node metastases
• Application of the grading system irrespective of histologic subtype is recommended