

DIAGNOSTIC IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY TESTING IN BELGIUM



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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ASA	Acetylsalicylic Acid
CK-BS	Broad Spectrum Cytokeratin
CKD	Chronic Kidney Disease
CMV	Cytomegalovirus
DGEC	Medical Evaluation and Control Service
EDS	Ehlers Danlos Syndrome
EM	Electron Microscopy
ENT	Ear, Nose and Throat (specialist)
EPS	Permanent Sample
GI	Gastrointestinal
GN	Glomerulonephritis
H&E	Haematoxylin and Eosin
HLA	Human leukocyte antigen
IEL	Intraepithelial Lymphocytes
IF	Immunofluorescence
IgA	Immunoglobulin A
IHC	Immunohistochemistry
IMA- AIM	InterMutualistic Agency
LM	Light microscopy
mIg	monoclonal Immunoglobulin
MOC	Multidisciplinary Oncology Consultation
MSI	Microsatellite Instability
NIHDI/INAMI/RIZIV	National Institute for Health and Disability Insurance
NSAID	Non-Steroidal Anti Inflammatory Drug



PCD	Primary Ciliary Dyskinesia
PGNMID	Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits
PUD	Peptic Ulcer Disease
RCD	Refractory Coeliac Disease
RUT	Rapid Urease Test
SECM	Service d'Evaluation et de Contrôle Médicaux
TTG-IgA	Tissue Transglutaminase IgA
UBT	Urea Breath Test



■ SCIENTIFIC REPORT

1 INTRODUCTION AND SCOPE

1.1 Aim and scope

This report aims to address a request by the NIHD (National Institute for Health and Disability Insurance) for KCE to provide a thorough overview on how immunohistochemistry (IHC) and electron microscopy (EM) diagnostic investigations are currently used in Belgium and how this use has evolved over time. It also provides a review of the clinical evidence linked to their use in the most common indications.

Two research questions were considered:

- First, what are the main indications for diagnostic IHC and EM investigations, and under what circumstances are these performed in Belgium?
- Second, what evidence exists on the efficacy of IHC and EM diagnostic investigations for the most common indications identified during the analyses performed to answer the first research question?

The focus of this report is limited to the diagnostic use of IHC and EM. We excluded pharmaco-predictive IHC tests for targeted oncological therapy from the scope of our research, since the Belgian Cancer Centre already produced detailed, tumour-specific workflows^a indicating the sequence of molecular tests to be carried out to ensure good clinical practice. The authors of this report are conscious that the distinction between diagnostic and prognostic investigations is not always clear, since diagnostic information, most often, also provides prognostic guidance. Nevertheless, a pragmatic decision to focus on diagnostic testing (based on current Belgian nomenclature) was taken to avoid, as much as possible, a duplication of efforts and limit the scope of our research. A description of the specific antigens included in the nomenclature codes for pharmaco-predictive IHC testing is presented in section 1.2.2 for information.

^a <https://www.compermed.be/en/workflows>



A detailed costing analysis was out of the scope of this project. Nevertheless, expenditure estimates, from the perspective of the NIHDI, are offered in the first part of our data analyses for a more comprehensive contextual description. In addition to this, in the literature reviews, if any of the included SRs or guidelines mentioned costs, a brief description is offered for completeness.

1.2 Immunohistochemistry testing

Immunohistochemical testing is a technique used in pathology and biology which aims to identify and visualise proteins (antigens or biomarkers) in tissue samples in order to report diagnostic information to the medical prescriber. It is a valuable tool for understanding the molecular and cellular components of biological specimens. It involves using antibodies, specific to a particular epitope of the protein of interest. These antibodies are tagged with a detectable marker, so that when applied to a tissue sample, they bind to their target proteins when present, allowing their visualization and facilitating their analysis.

Although the principle of IHC exists since the 1930s, it was only in 1941 that the first IHC study by Coons et al.¹ was performed. Over the years, this testing technique has evolved and improved noticeably, making it a powerful tool to better understand disease processes and facilitate patient diagnosis and treatment decisions. As a consequence, the use of these tests has increased significantly and their budgetary impact has followed the same trend.

1.2.1 Common fields of use

IHC is a versatile tool, not just in the clinical setting, but also in research, which provides valuable information about the distribution and quantities of proteins in various tissues. At present, IHC testing is used in different fields, particularly in oncology, to determine the origin of tumours, identify specific cancer markers, or classify tumours based on their molecular characteristics. Other areas of interest include the diagnosis and study of neurological disorders or infectious diseases.²

1.2.2 Reimbursement of IHC testing in Belgium

Two types of IHC staining are currently reimbursed in Belgium:

Diagnostic IHC testing is reimbursed by the NIHDI since the first of April 1985 under nomenclature codes 588070-588081. These codes can be used a maximum of 4 times per sampling, and reimbursement cannot be accumulated for the same antigen. On the first of April 2021, the NIHDI modified the existing nomenclature to create separate codes for IHC diagnostic testing on special biopsies, allowing a higher number of stains per sampling for the investigation of: central nervous system tumours, soft tissue tumours, mesothelial tumours or when investigating non-oncological pathologies of the kidney or liver, (nomenclature codes 587075-587086); and for the investigation of lymphoid tumours, (nomenclature codes 587090-587101). For these new nomenclature codes, a maximum of 8 IHC stains per sampling can be used (instead of 4), but each stain can only be used once per year.

Pharmaco-predictive IHC testing are reimbursed by the NIHDI since the first of July 2009, under nomenclature codes 588976-588980. Between 2009 and 2017, five specific antigens could be used (i.e. epidermal growth factor receptor (EGFR), oestrogen receptor (OR), progesterone receptor (PR), cerb-B2/her2/neu, and C-kit/CD117). Since the first of April 2021, a different set of 7 antigens are reimbursed (OR, PR, Cerb-B2/her2/neu, PD-L1, ALK1, ROS1 and TRK). Pharmaco-predictive codes can be used only for one sampling per year, for a maximum of 10 stains. Diagnostic and pharmaco-predictive codes are not cumulative for the same antigens.

Table 1 offers a description of the nomenclature codes.

**Table 1 – Belgian nomenclature codes for IHC tests**

Nomenclature code	Description	Reimbursement per stain in 2024
588070 588081	- Immunohistochemical examination for detection of antigens, by antiserum	32.15€
588976 588980	- Immunohistochemical examination for the detection of pharmaco-predictive or prognostic biomarkers in a tumour, by antiserum, in the context of prescribing tumour-specific medication	96.44€
587075- 587086	Immunohistochemical examination for the detection of antigens in the diagnostic investigation phase of a tumour of the central nervous system, a soft tissue tumour, a mesothelial tumour or in the investigation of non-oncological pathology of kidney or liver, by antiserum.	32.15€
587090- 587101	Immunohistochemical examination for the detection of antigens in the diagnostic investigation phase of a lymphoid tumour, by antiserum.	32.15€

Translated from :

https://www.inami.fgov.be/SiteCollectionDocuments/nomenclatureart32_20210701_01.pdf
(FR)

https://www.riziv.fgov.be/SiteCollectionDocuments/nomenclatuurart32_20210401_20210630_01.pdf (NL)

1.3 Electron Microscopy testing

EM is an imaging technique using a focused beam of electrons to obtain high-resolution images of the ultrastructure of biological specimens and other materials, therefore allowing a much finer level of detail than traditional light microscopy.

EM was first introduced by Ernst Ruska and Max Knoll in the 1930s, with the first functional electron microscope dating from a decade later.³

Over the years, EM has evolved with advances in electron sources, lenses, detectors, and sample preparation techniques. Such advances led to even higher resolution and imaging capabilities.

1.3.1 Common indications

EM is used to study the fine structure of cells and subcellular components. In medical diagnostics, it can help identify ultrastructural abnormalities in tissues, aiding in the diagnosis of certain diseases such as kidney or neuromuscular diseases, or viral, bacterial or parasitic infections amongst others.⁴⁻⁷ It is also commonly used in different fields of scientific research.

1.3.2 Reimbursement of EM diagnosis in Belgium

EM is reimbursed by the NIHDI since the first of April 1985, under nomenclature codes 588114-588125.



Table 2 – Nomenclature of EM tests

Nomenclature code	Description	Reimbursement per test in 2024
588114-588125	Anatomo-pathological examination with an electron microscope, regardless of the technique/s used, and regardless of the number of samples taken	396.48€

Translated from :

https://www.inami.fgov.be/SiteCollectionDocuments/nomenclatureart32_20210701_01.pdf (FR)

https://www.riziv.fgov.be/SiteCollectionDocuments/nomenclatuurart32_20210401_20210630_01.pdf (NL)

2 THE USE OF DIAGNOSTIC IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY IN BELGIUM

This section explores the use of diagnostic IHC and EM investigations in Belgium over the last years (2012-2021) from a health care payer perspective.

2.1 Methods

The methods used in this research can be broken down into three different phases. A summary table listing all sources used, the period they cover and the variables they include is presented at the end of this section (see Table 3).

2.1.1 Phase 1 – Analysis of data from the NIHDI

2.1.1.1 NIHDI annual data on use based on nomenclature code registration (Doc N)

Four times per year, the NIHDI provides KCE with aggregated annual data per nomenclature code on expenditure covered by the compulsory health insurance. These anonymised data are directly captured by the NIHDI. For this study, this source of data was consulted to extract information on the number and cost of IHC and EM examinations (and medical procedures leading to those investigations), that were reimbursed over the period 2012-2021.

2.1.1.2 Anonymised data from pathology reports

Given the lack of registration via the existing nomenclature codes of the indications for which IHC and EM are used, other available sources were explored. The aim was to provide an insight into the specific medical fields in which these investigations are most commonly used in Belgium. This was considered an important step to better focus the efforts of our research, and



more specifically, the literature reviews needed to be performed as part of this study.

For this purpose, pathology reports from all laboratories were requested by the Medical Assessment and Control Service of the NIHDI.^b

- For EM testing, pathology reports for all such investigations performed in 2019 were summarised internally by the NIHDI and the basic anonymised information was captured in a spreadsheet which was shared with the KCE research team. This offered a good (although static) view of the use of these tests in that year.
- For IHC testing, given the high number of tests carried out every year in Belgium, a sample of tests performed in 2019 was drawn and the relevant information from the pathology reports was anonymised and summarised in a further spreadsheet which was also shared with the KCE team.

Sampling (for IHC only) was performed by the NIHDI: A list of unique patients on which IHC staining was performed was made. The information was first sorted by IHC prescriber (from most to least frequent prescriber), then by date of testing, followed by patient number. Once this was done, every 150th “unique” patient was selected and the specific IHC staining performed for each of these unique patients was included in the sample. For the 67 laboratories in activity in 2019, 2707 patients were identified, if additional IHC stains were carried out on a different date, these were also captured as a different “contact” (i.e. sampling session). A total of 3782 contacts were included in the sample.

The (anonymised) data on all antigen combinations tested were summarized per indication and exported to an excel sheet shared with the KCE research team for analysis. When no indication was mentioned in the reports, a proxy for this was derived from the body site of the biopsy and the stains used in that specific case. A preliminary classification was performed by the NIHDI, but it was thought to be important for the KCE team to look at the data in some detail, to ensure 1) that the best and more accurate classification

would be used for the purpose of the research here presented and 2) that all relevant data on use had been extracted. In order to minimise the time and resources required to perform such a task, a check was performed. Ten (anonymised) pathology reports were analysed by two clinical experts from KCE, and discussed within the KCE team. If there was an agreement regarding the classification performed and no additional informative data was identified during this test, the spreadsheet received from the NIHDI would be used as such. On the contrary, a detailed review of the (anonymised) pathology reports would be carried out internally at KCE, prior to completing the data analysis.

Data extrapolation was performed using a multiplication factor. This was calculated as the ratio of the number of contacts, captured in the sample of pathology reports drawn by the NIHDI and the number of contacts captured in the permanent sample for the year 2019 (see point 2.1.2). A 95% confidence interval was calculated by taking the 2.5% of the multiplication factor as upper and lower bounds. This gives a crude (over) estimation of the number of IHC stains for the year 2019 due to the two different steps involved in the extrapolation (i.e. the use of extrapolated EPS data, followed by the use of the sample drawn by the NIHDI).

2.1.2 Phase 2 – Analysis of data from the permanent sample

IMA–AIM (Inter-mutualistic Agency) is a non-profit organisation that manages and analyses information on all reimbursements related to the compulsory health insurance in Belgium, collected by the Belgian sickness funds on all insured citizens. These data cover all reimbursed services (consultations, pharmaceuticals, diagnostic and therapeutic procedures) and some patient socio-demographic characteristics as well as social security related data, to the extent they influence reimbursement.

IMA–AIM composes and maintains a substantive sample called the Permanent Sample (EPS). The EPS is composed of randomly drawn members of the Sickness Funds, stratified by age and gender, approximately 1 in 40 for members younger than 65, and 1 in 20 for

^b Dienst voor geneeskundige evaluatie en controle (DGEC) - Service d'évaluation et de contrôle médicaux (SECM)



members aged 65 and older. The purpose of the larger sample in the 65 years and older is to increase the precision for this group, which has relatively higher health expenditure, and ensure the older age groups do not become too small. The sample is longitudinal and contains data since 2002. Every year, an additional sampling, similar to the original one, compensates for members lost to follow-up due to decease or other reasons.

These EPS data in the context of our research were mainly used to explore the extent to which important pathologies for which diagnostic IHC testing is currently performed in Belgium could be identified and studied. Moreover, general trends in IHC use were also studied. Data on EM use was not extracted, given that these is a relatively rare testing method, and thus, the representativity of such data in the permanent sample, would be compromised.

2.1.2.1 Selection of data:

The analysis of the data from the permanent sample was aimed at better understanding how diagnostic practices in the most common indications for IHC testing in Belgium have evolved overtime. In addition to this, an identification of other tests and interventions often performed in patients diagnosed by means of the IHC tests was also pursued. Finally, a more detailed analysis of IHC diagnosis in gastrointestinal (GI) biopsies (and more specifically in *H. pylori* and coeliac disease) was attempted, by identifying patients having had a gastric biopsy, followed by IHC testing or a *H. pylori* or coeliac disease-specific non-invasive test.

Three different selections were performed.

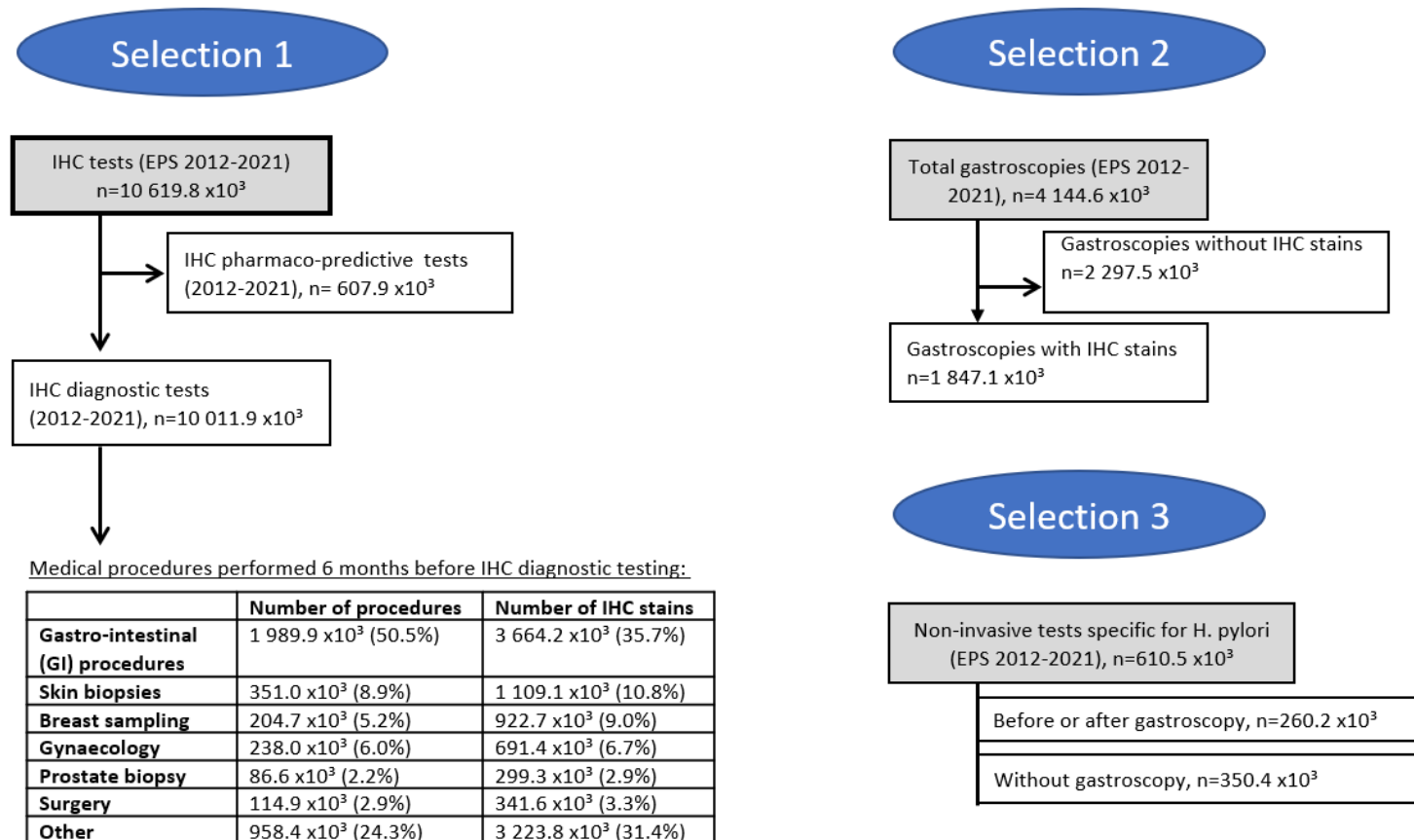
- First, a more general selection, where the starting point was all IHC tests performed during the years 2012-2021 (see Table 1 for nomenclature codes). From those, all the medical acts completed over the prior 6 months, were selected, in order to identify the medical procedures leading to a biopsy. Thus, for every IHC test, possible medical procedures carried out during the previous 6 months were looked at. Other information such as standard histochemical testing (i.e. Hematoxylin and Eosin (H&E) staining), prescriber or number of IHC stains were also investigated.
- Second, the starting point was all the gastroscopies (see the appendix for nomenclature codes) performed during the years 2012-2021. From those, all medical acts performed 6 months before and after the intervention were captured, in order to find (1) if histology followed the gastroscopy (i.e. a biopsy was taken), (2) if pre-gastroscopy or post-gastroscopy tests were realized, and if so which ones, and finally, (3) if any specific treatment for *H. pylori* was initiated.
- Third, the starting point was all the different non-invasive tests specific for *H. pylori* (see the appendix for nomenclature codes) during the years 2012-2021. From those, all the medical acts performed during the 6 months before and after were studied, in order to find (1) if there was a gastroscopy carried out before or after the test, (2) if there were histology or IHC staining applied (i.e. a biopsy was taken during the gastroscopy), and (3) if any specific treatment for *H. pylori* was initiated.

These different selections are illustrated in Figure 1 as flow charts, with the different numbers of procedures and IHC tests included in our EPS data analyses for the period 2012-2021.

Regarding medical treatment, the following pharmaceutical products were considered in our analyses: Proton-pump inhibitors (PPIs), antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs) and Bismuth (i.e. common treatment for *H. pylori*). Stratification was made according to age.



Figure 1 – Flow charts of the selection of data in the EPS for 2012-2021



EPS is limited to captured to nomenclature codes and is therefore limited to reimbursed services



2.1.3 Phase 3 – Survey to all Belgian laboratories

A survey was sent to all Belgian laboratories to: 1) confirm the most common indications for which diagnostic IHC staining or EM tests are performed, and 2) understand in which circumstances these tests are considered appropriate or needed.

With that in mind, questions were raised on the five most frequent indications on which IHC testing was performed, and the three most common for EM testing, in the years 2019 and 2022. In addition to this, a request to mention guidelines, protocols or any other decision-making tool or base was made for each frequent indication. Moreover, questions on the overall number of diagnostic IHC stains performed in the years 2019 and 2022 were also posed. A conscious decision was made to exclude the years of the COVID-19 pandemic in this survey, since any information gathered for that period would not be representative of standard practice.

The overall number of EM tests performed in 2019-2022 was not requested, since data of the NIHDI captured all EM investigations and not just a sample, and therefore offered a good overview for EM use in 2019. For the year 2022, the small number of laboratories performing EM, and the existence of laboratory-specific indications, would make it difficult for a voluntary survey to offer a relevant picture on the numbers.

Given that all other data sources were limited to reimbursed use, we asked survey participants how many of the diagnostic IHC stains were not paid for by NIHDI and the main reasons why they were not covered.

A draft of the survey was shared with the presidency of the Belgian Commission of Anatomic Pathology, as well as an expert in the field, who collaborated with the KCE research team by giving input and helping to adapt the survey to ensure optimal understanding from the responders perspective, while keeping the survey as short as possible, focusing purely on key questions that could not be explored by any other means (i.e. administrative data analyses or reviews of the literature).

Two separate surveys (Limesurvey), one for IHC and one for EM, available in both French and Dutch, were sent with the help of Siensano^c on behalf of the presidency of the Belgian commission of anatomic pathology to all laboratory directors. Participation was voluntary and participants had one month to respond. A copy of these surveys is presented in appendix.

The answers to this survey were expected to offer an important overview on use and decision-making in this field, and facilitate a correct and more complete interpretation of the data analyses and literature reviews carried out as part of this research. They also targeted the identification of any existing guidelines, protocols or other important documentation worthwhile investigating and unlikely to be picked up via a literature search. Responses to the survey were anonymous and their analysis and reporting was pursued, and here presented, on an aggregated manner.

Table 3 offers a summary on the sources consulted, the period they cover and the variables they include.

^c <https://www.sciensano.be/en>



Table 3 – Overview of sources consulted for our data analyses

Phase	Type of data	Period covered	Available variables
I. Analysis of data from the NIHDI	Aggregated annual expenditure data	2012-2021	Aggregated numbers and costs for reimbursed services. Used to extract the N. of IHC and EM tests performed, as well as the expenditure linked to their use.
	Anonymised data from pathology reports	2019	Patient ID, laboratory ID, specialty of the prescriber, body site for the biopsy, specific IHC stain performed, number of stains billed and amount reimbursed.
II. Analysis of data from the permanent sample	Selection 1: All IHC stains reimbursed Medical procedures leading to a biopsy (6 months before IHC staining).	2012-2021	Number of IHC stains per procedure, number of procedures leading to an IHC stain, prescribers of IHC and laboratories.
	Selection 2: All gastroscopies reimbursed Any histology and IHC staining performed on the following 6 months after a gastroscopy. Any pre-gastroscopy or post-gastroscopy tests, and treatment (6 months before and after).	2012-2021	Number of gastroscopies, number of gastroscopies leading to an IHC stain, prescribers of gastroscopies, hospitals performing the gastroscopies, number of pre-gastroscopy or post-gastroscopy tests (i.e. non-invasive tests for <i>H. pylori</i> , non-invasive test for coeliac disease), treatment (specific for <i>H. pylori</i>).
	Selection 3: Non-invasive tests for <i>H. pylori</i> Gastroscopies performed and/or <i>H. pylori</i> treatment on the 6 months before and after a non-invasive <i>H. pylori</i> test.	2012-2021	Number of non-invasive tests for <i>H. pylori</i> , number of associated gastroscopies, with histology and IHC, and specific treatment for <i>H. pylori</i> .
III. Survey to all Belgian laboratories		2023	IHC consumption for the year 2019 and 2022, most common indications for which diagnostic IHC staining or EM tests are used



2.1.3.1 Limitations

All the available administrative databases consulted for our analyses are based on reimbursement data. The overview is therefore, given from a payer perspective (i.e. the NIHDI) and excludes a detailed outline on private IHC use.

Regarding the data extracted from the pathology reports, we cannot exclude the sampling performed to have missed some rare IHC indications or antigens. Nevertheless, the aim of the exercise was to highlight the most frequent indications and use for these tests, so an exhaustive list of indications and antigens was not considered necessary in this context.

To overcome the lack of information on indications, the procedures carried on in the 3 months preceding the IHC test were identified in the EPS data set. All IHC stains prescribed by the same prescriber within a 3 months of the first IHC were grouped and considered to be part of the same investigation. An exception was made for IHC requested by a different prescriber. Despite the efforts, the case studies on *H. pylori* and coeliac disease suffer from great uncertainties, and proxies needed to be used in multiple occasions throughout our analysis (e.g. for the identification of positive *H. pylori* cases). This is clearly explained in the relevant parts of the results section. In addition to this, some figures presented in this report required the combination of different sources of information, which may in turn pose some limitations on the extrapolations made. Whenever possible, the available sources used were tested for consistency.

Our survey addressed to all Belgian laboratories was voluntary, and relied on self-reported data. Its results are therefore subjected to bias. In particular, the participation bias may hamper the representativeness of results. Moreover, its anonymous nature impedes to track the background of the respondents which may in some cases consists of pathologists and in others of laboratory managers. The extent to which answers represent the general use of IHC and EM testing in the different laboratories, or the use by a specific pathologist, remains unknown. The survey was sent to all laboratories with an agreement for reimbursement by the NIHDI. The existence of other laboratories performing such tests privately was not explored.

2.2 Results: Immunohistochemistry staining

2.2.1 Comparison of data sources: EPS (extrapolated data) and Doc N (NIHDI)

A concordance check was performed comparing the real observed numbers of IHC stains published by the NIHDI data with the extrapolated numbers of IHC tests found in the EPS data. The results from this check can be seen in Table 4 and confirmed a good correspondence of the data, suggesting that the extrapolated EPS data offered a good estimate of the number of IHC stains and thus, could be further analysed to describe trends and use of IHC stains in more detail.


Table 4 – Concordance of the EPS extrapolated data with NIHDI data of IHC stains (x10³)

Nomenclature codes	Period 2012-2021		Year 2019		Year 2021	
	IHC stains in EPS (95% IC)	IHC stains in Doc N	IHC stains in EPS (95% IC)	IHC stains in Doc N	IHC stains in EPS (95% IC)	IHC diagn stains in DocN
588070	7046.4 [7008.4;7084.4]	7062.8	862.3 [844.7;879.9]	852.0	996.2 [977.1;1015.3]	986.8
588081	2981.9 [2950.8;3012.9]	3016.6	356.2 [344.5;367.9]	342.3	327.4 [316.2;338.6]	338.0
588976	338.3 [327.9;348.7]	338.8	37.4 [33.8;40.9]	36.3	49.7 [45.8;53.6]	48.7
588980	207.9 [199.9;215.8]	211.3	22.6 [19.9;25.2]	19.5	23.8 [21.1;26.4]	24.4
587075	13.2 [9.9;16.6]	9.7	-	-	13.2 [9.9;16.6]	9.7
587086	14.3 [11.0;17.6]	15.8	-	-	14.3 [11.0;17.6]	15.8
587090	23.0 [18.5;27.4]	23.3	-	-	23.0 [18.5;27.4]	23.3
587101	12.4 [9.2;15.6]	12.2	-	-	12.4 [9.2;15.6]	12.2

Source: EPS, NIHDI, nomenclature codes 587075, 587086, 587090, 587101 only available since 01/04/2021.

2.2.2 Histology and IHC

The NIDHI looked at the number of IHC performed after a H&E histological examination. Belgian nomenclature categorises histologic procedures in 7 groups (see Table 5), according to dimension of the sampling and the type of tissue (biopsies, surgical pieces or cytologic sampling).

Regular biopsies are the most important sampling for histologic procedures, and data show that in 2019, IHC was performed on 34% of cases.

**Table 5 – Number of IHC performed after either a histologic or a cytologic procedure in 2019 (Source: NIHDI)**

	N. of Histology performed	N. of Histology with diagnostic IHC	% of Histology with diagnostic IHC
Regular biopsy	1 039 884	355 588	34%
Mini biopsy	24 244	444	2%
Super biopsy	80 984	56 997	70%
Small macroscopy	87 598	28 414	32%
Large macroscopy	39 697	18 217	46%
Mini cytology	43 616	2 331	5%
Regular cytology	111 111	30 127	27%

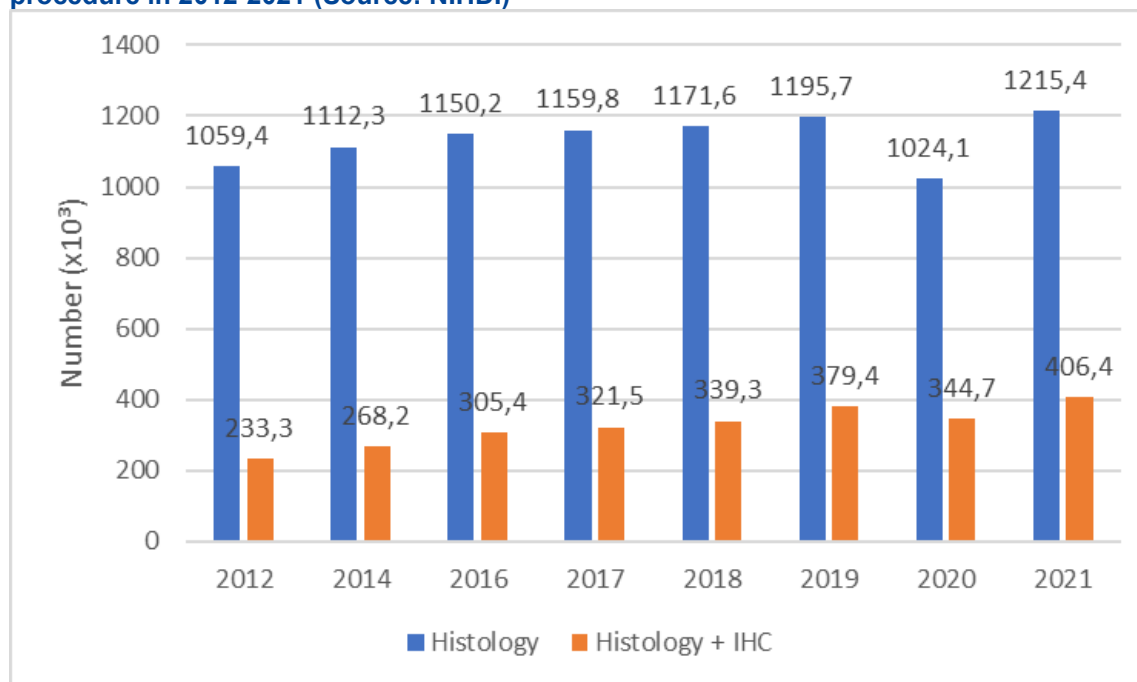
Source: NIHDI Histologic or cytologic procedures: nomenclature codes : 588011, 588022, 588232, 588243, 588254, 588265, 588276, 588280, 588291, 588302, 588394, 588405, 588416, 588420, diagnostic IHC: nomenclature codes : 588070-588081.

The same exercise was performed for the period 2012-2021. The proportion of IHC performed biopsies increased over the years (from 22.0% in 2012 to 33.4% in 2021). This increase reflects a higher growth in IHC staining, compared to histologic procedures. Based on the absolute numbers presented in Figure 2, we calculated that the increase in IHC stains was 74.2%, which is significantly larger than the increase of 14.7% of histological procedures. For the year 2019, we can see that in 32% of cases IHC is performed on these samples, which appears to correspond well to the NIHDI data (although not an exact match).

Overall, an increase in histology of 13% between 2012 and 2019 was seen, while a higher increase of 63% was seen in IHC associated with histology. It is important to note that nomenclature codes for “regular biopsies” are at present, independent of the number of individual biopsies (i.e. captured in the administrative database as one biopsy regardless of the amount of samples retrieved in a sampling session). Therefore, we cannot exclude that over time, the number of biopsies per case may have changed. As a consequence, some uncertainty in the relative increases (slower increase in the number of regular biopsies analysed by histology, than in IHC stains) should be recognized.



Figure 2 – Number of regular biopsies (x10³) on which IHC was performed after a histologic procedure in 2012-2021 (Source: NIHDI)



Source: NIHDI aggregated data: Includes regular/mini/super biopsies (nomenclature codes: 588011, 588022, 588232, 588243, 588254 and 588265)

2.2.3 General evolution in use of IHC stains

Table 6 and Figure 3 show the number of IHC stains to have grown each of the studied years, with the exception of 2020, the year of the COVID pandemic, where a small decrease is observed. The associated public expenditure follows the same trend. The growth in IHC expenditure between 2012 and 2019 (excluding the years of the COVID pandemic) was 74.0%. This represents more than double the growth in the expenditure of the total anatomic-pathological expenditure over that same period (34.1%).

In 2012, IHC stains represented 15.6% of the total anatomic-pathological expenditure. This proportion grew up to 20.2% in 2019.

Diagnostic IHC stains represent the large majority of all IHC stains carried out (over 94% of all reimbursed IHC stains), and this remains the case throughout the study period.

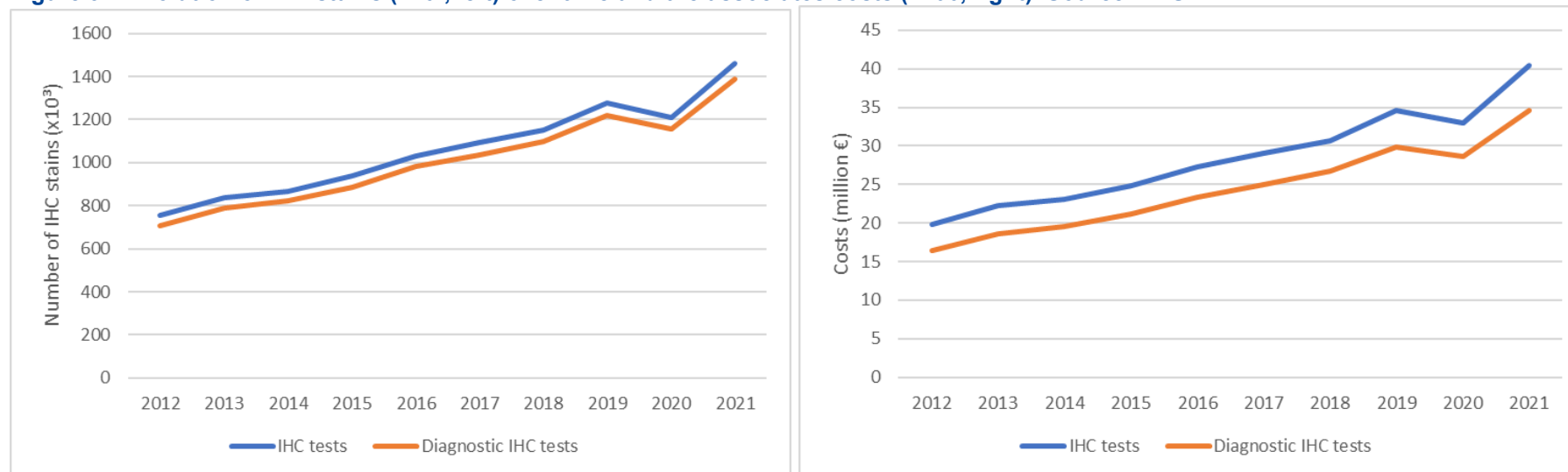
**Table 6 – Evolution of IHC stains 2012-2021 (source: EPS)**

Year	IHC stains: Diagnostic and pharmaco-predictive IHC stains		Diagnostic IHC stains	
	Number of stains (x10 ³ €) (95% IC)	Costs (x10 ⁶ €) (95% IC)	Number of stains (x10 ³ €) (95% IC)	Costs (x10 ⁶ €) (95% IC)
2012	752.9 [736.7;769.1]	19.9 [19.4;20.3]	705.8 [690.1;721.5]	16.4 [16.1;16.8]
2013	838.4 [821.3;855.6]	22.3 [21.8;22.7]	787.7 [770.9;804.4]	18.5 [18.1;18.9]
2014	868.5 [851.1;885.9]	23.1 [22.6;23.6]	820.6 [803.6;837.6]	19.6 [19.2;20.0]
2015	936.8 [918.6;955.1]	24.9 [24.3;25.4]	886.0 [868.2;903.8]	21.1 [20.7;21.6]
2016	1032.3 [1013.1;1051.5]	27.2 [26.7;27.8]	980.0 [961.2;998.8]	23.4 [22.9;23.8]
2017	1091.5 [1071.8;1111.1]	29.0 [28.5;29.6]	1036.7 [1017.5;1055.9]	24.9 [24.5;25.4]
2018	1149.4 [1129.2;1169.7]	30.7 [30.1;31.3]	1096.9 [1077.0;1116.7]	26.7 [26.2;27.2]
2019	1278.5 [1257.3;1299.7]	34.6 [33.9;35.2]	1218.5 [1197.8;1239.3]	29.9 [29.4;30.4]
2020	1211.5 [1190.6;1232.4]	33.0 [32.4;33.7]	1156.1 [1135.5;1176.6]	28.7 [28.2;29.2]
2021	1460.0 [1436.7;1483.2]	40.4 [39.8;41.1]	1386.5 [1363.6;1409.3]	34.6 [34.0;35.2]

Source: EPS, Diagnostic correspond to nomenclature codes 588070, 588081, 587075, 587086, 587090, 587101, and pharmaco-predictive IHC stains correspond to nomenclature codes 588976, 588980.



Figure 3 – Evolution of IHC stains ($\times 10^3$, left) over time and the associates costs ($\times 10^6$, right). Source: EPS



Given that the focus of this report is on diagnostic IHC stains (i.e. nomenclature codes 588070 and 588081), the following sections of our data analyses exclude the pharmaco-predictive IHC stains (i.e. nomenclature codes 588976-588980).



2.2.3.1 Evolution of IHC staining per type of institution

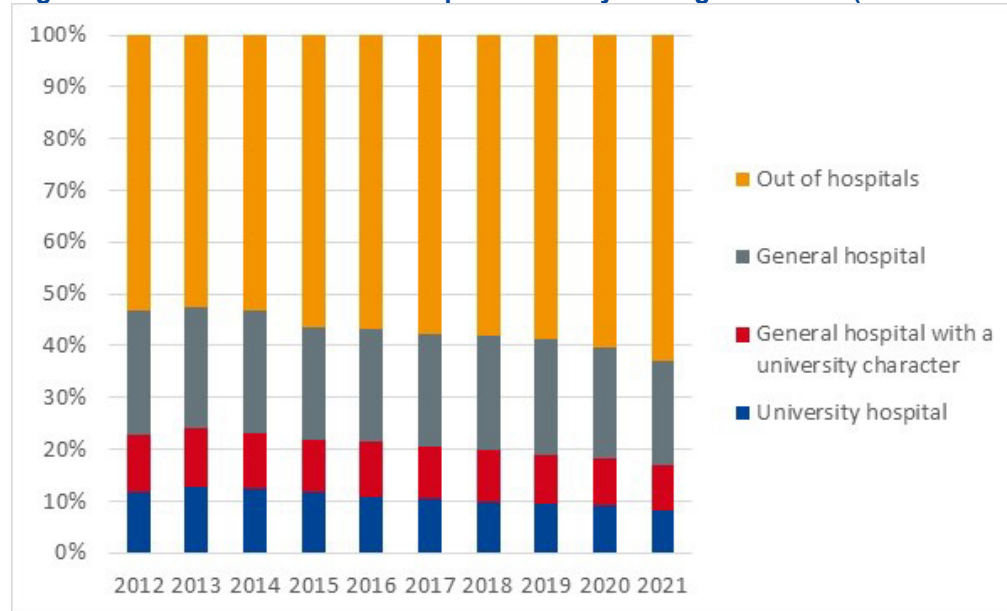
Laboratories are independent structures, not necessarily attached to a hospital. They need to have the agreement 32^d to be able to receive reimbursement for the performance of these anatomical pathology tests. Figure 4, shows that only a small proportion of laboratories were associated to a hospital in 2019.

When looking at the number of IHC stains performed per type of institution^e (see Figure 4), a small annual increase is seen in hospitals (university hospitals 3.3%, general hospitals with a university character 4.4%, and

general hospitals +5.6%). Conversely, for IHC stained performed out of hospital the annual increase is more marked (+9.4%).

Laboratories evaluate sampling coming from different settings, whether from hospitals or from the community (i.e. out of hospital). Hospital samples represented 41.4% of all samples in 2019 (9.6% from university hospitals, 9.3% from general hospitals with a university character and 22.5% from general hospitals).

Figure 4 – Evolution of IHC stains performed by setting 2012-2021 (source: EPS)



^d Royal decree 05/12/2011 available at https://www.gbs-vbs.org/fileadmin/user_upload/Wetgeving/AR_05.12.2011_Agrement_des_laboratoires_d_anatomie_pathologique.pdf

^e <https://www.healthybelgium.be/en/key-data-in-healthcare/general-hospitals/organisation/types-of-hospitals>



Conclusion – General use and trends for IHC in Belgium

- Reimbursement of IHC stains is grouped in two categories: diagnostic and pharmaco-diagnostic. The second group is more cancer oriented. The focus of this study is on diagnostic IHC stains (>94% of all reimbursed IHC stains in 2019).
- The use of diagnostic IHC staining on regular biopsies increased by 63% between 2012 and 2019, while the use of histological staining (H&E) for regular biopsies also increased, but at a lower rate (+13% between 2012 and 2019). However, the rate of growth for the latter, is subject to some uncertainties, given a lack of registration of data on the exact number of biopsies taken (only 1 biopsy per contact is registered).
- By setting, the proportion of IHC stains in biopsies taken in hospitals has decreased when compared with the proportion in biopsies taken out of hospital.

2.2.4 Indications for IHC testing.

2.2.4.1 Analysis of pathology reports from laboratories

The sampling of IHC for the year 2019 carried out by the NIHDI is presented by contact (i.e. sampling session). Patients with multiple sampling sessions (IHC staining performed on multiple dates) could also be identified, using a specific patient number.

For each contact, information on the main body site examined was extracted, together with the number of antigens tested for the 2 707 patients identified. The overall number of contacts, considering additional IHC staining performed in different dates in the selected population, was 3 782.

- Main body sites and number of stains:

Overall, 13 body sites were identified in the NIHDI data. The five sites with the highest number of examinations were GI, (38.2%), breast (15.0%), skin (13.9%), cervix and other gynaecology. To be noted: The indications for IHC testing were divided by the NIHDI in “oncological” (clearly oncological) and “non- oncological” (a mix of non-oncological indications and indications where an oncological disease may need to be excluded). For pragmatic reasons, this classification has been kept throughout this report.

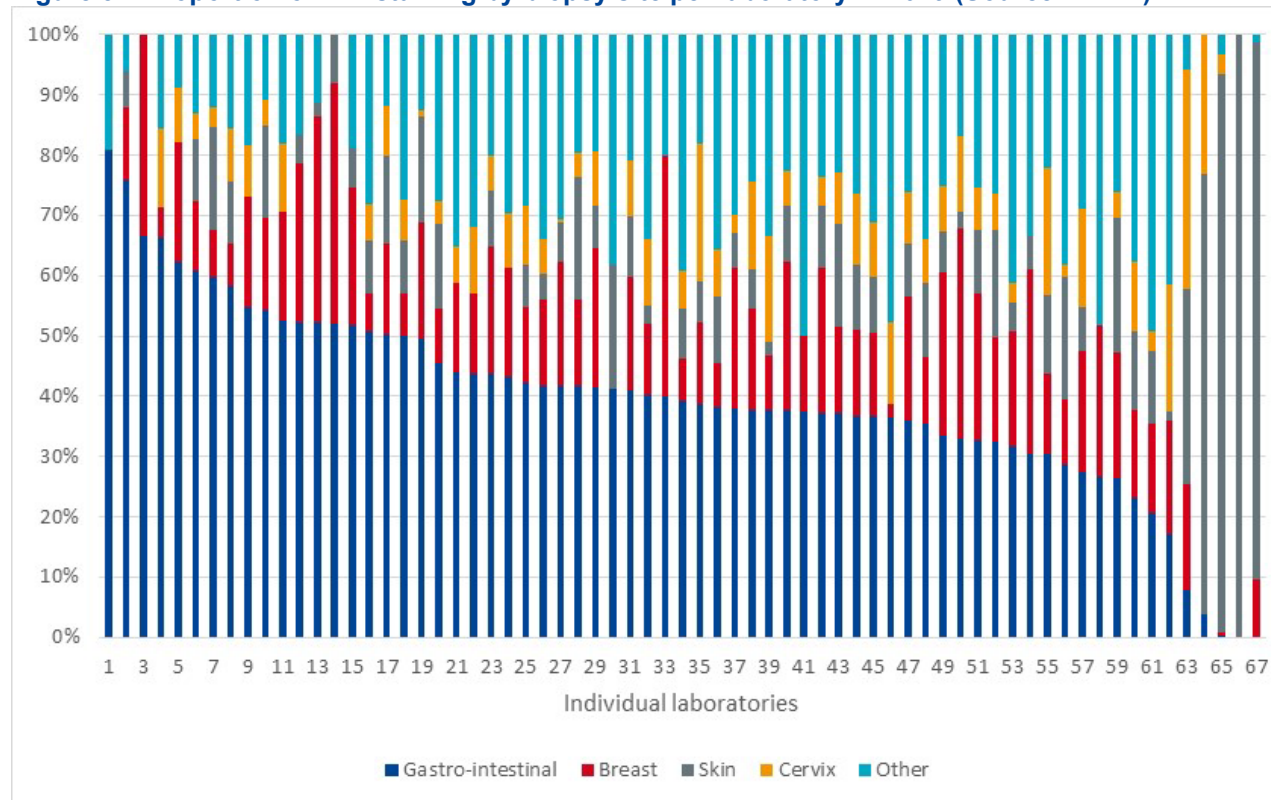
In some pathology reports, an indication could not be identified (n=144 reports), while 10 reports proved to be duplicates. Table 7 summarises these findings.


Table 7 – Body sites for IHC stains in 2019 (Source: NIHDI)

	Extrapolation of number of IHC stains (x10 ³)	Extrapolation total expenditure (Million €)	Most frequent stains
Gastro-intestinal	466.1 [454.5;477.8]	11.62 [11.33;11.91]	<i>H. pylori</i> , CD3, P53, CMV, Ki67, CK
Breast	182.6 [178.0;187.2]	4.63 [4.52;4.75]	ER, PR, HER2, Ki67, P63, E-cadherine
Skin	170.1 [165.8;174.3]	4.06 [3.95;4.16]	MelanA, Ki67, HMB45, P16, Sox-10
Cervix	90.9 [88.6;93.2]	2.19 [2.14;2.25]	P16, Ki67, <i>H. pylori</i>
Lung	63.1 [61.6;64.7]	1.54 [1.50;1.58]	TTF1, PD-L1, CK7, P40, ROS -1
Gynecology	54.8 [53.5;56.2]	1.29 [1.26;1.32]	p53, ER, P16, Ki67, CD138
Prostate	47.3 [46.2;48.5]	0.91 [0.89;0.93]	P504S, P63, Ck34βE12
Hematology	33.2 [32.4;34.0]	0.84 [0.82;0.86]	CD20, CD3, CK
Urology	25.0 [24.4;25.7]	0.57 [0.56;0.58]	CK20, Ki67, CK7, p53, p63
Liver	22.0 [21.5;22.6]	0.57 [0.55;0.58]	CK7, CK20, CDX2
None/Duplicate/other	53.4 [52.0;54.7]	0.86 [0.84;0.88]	
Kidney	10.7 [10.5;11.0]	0.28 [0.27;0.29]	IgA, IgG, C3d, C4d, IgM
Total	1219.4 [1188.9;1249.9]	29.80 [29.06;30.55]	

A total of 312 different stains were registered. The most frequent stains are present by body site in an appendix. Briefly, *H. pylori* is the most frequent IHC stain (14.6% of all IHC stains performed in Belgium in 2019) and its use is limited to GI body sites. This is followed by Ki67 (8.0%) used in multiple body sites (breast, cervix, GI, skin, gynaecology, urology, lung, haematology, liver, prostate, cervix and others). Broad spectrum cytokeratin stains (CK) is the third stain most commonly used (4.5% of all IHC stains). It is also carried out in multiple body sites (breast, cervix, GI, skin, gynaecology, kidney, lung, haematology, liver, prostate, cervix and others).

As previously seen, In 2019, the number of IHC stains is the highest in GI biopsies. An analysis per laboratory was performed in order to assess variations in practices. For 80% of the laboratories, IHC staining for GI indications represented between 30% and 70% of all IHC stains. In two laboratories, IHC staining for GI indications was not carried out (see Figure 5).

**Figure 5 – Proportion of IHC staining by biopsy site per laboratory in 2019 (Source: NIHDI)**

IHC staining for *H. pylori* (incl. *H. pylori*-only, and combinations of *H. pylori* and other stains) is captured under GI biopsies. The data shown in Table 8, illustrates that IHC staining for *H. pylori* requires on average one stain only. Bearing this in mind, the total number of IHC stains for *H. pylori* can be estimated also for those cases in which this stain is performed in combination with other IHC stains, which can then be added to the number of *H. pylori*-only stains (i.e. 142.00 (*H. pylori*-only) + 72.7/2.9 (*H. pylori* &

CD3/CD4/CD8 (+other))+75.1/2.6 (*H. pylori* & other than CD3/CD4/CD8)=196.0). Such calculations show *H. pylori* to account for approximately 42.0% (i.e. 196.0/466.1) of all IHC stains performed in GI biopsies. A similar calculation shows that IHC staining most commonly used for coeliac disease (i.e. CD3, CD4 and CD8) accounts for 14.2% of all IHC stains undertaken in GI biopsies. The remaining (43.8%) include oncologic IHC tests, CMV, giardia, etc.



Table 8 – Analysis of GI indications in 2019 (source: NIHDI)

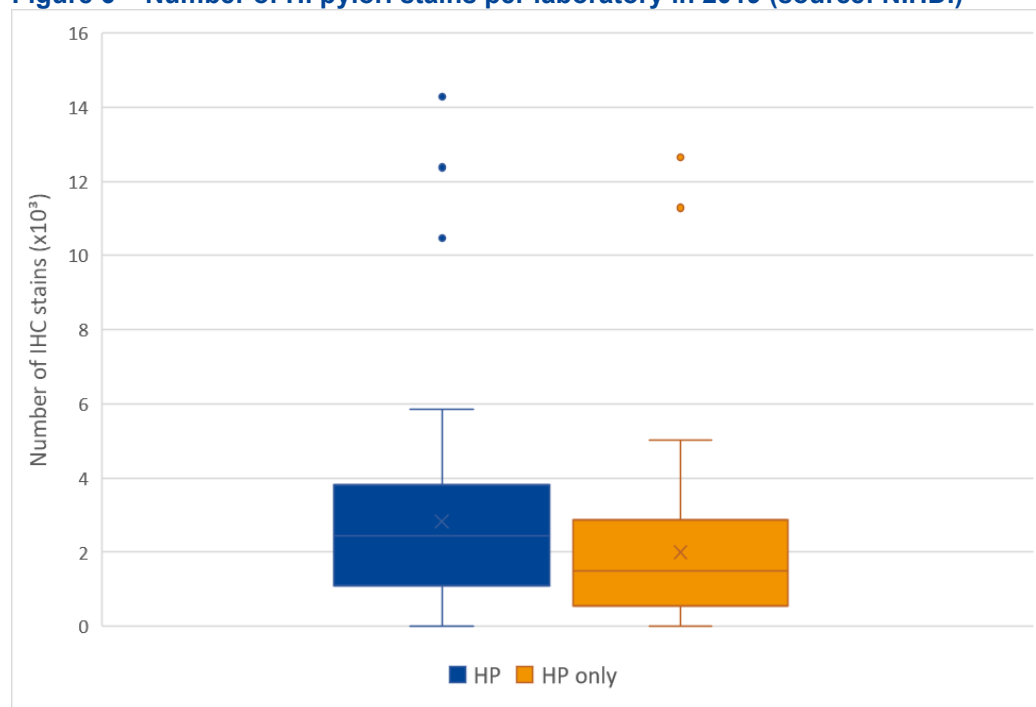
	Number of contacts	Mean N. of IHC stains/contact	Overall N. of IHC stains	Extrapolation of number of IHC stains (x10 ³)	Extrapolation of total expenditure (Million €)
Gastro-intestinal	1880	1.8	3426	466.1 [454.5;477.8]	11.62 [11.33;11.91]
- <i>H. pylori</i> -only	986	1,1	1044	142.0 [138.5;145.6]	3.49 [3.40;3.57]
- <i>H. pylori</i> & CD3/CD4/CD8 (+other)	182	2.9	534	72.7 [70.8;74.5]	1.79 [1.75;1.83]
- <i>H. pylori</i> & other than CD3/CD4/CD8	212	2.6	552	75.1 [73.2;77.0]	1.85 [1.80;1.90]
- CD3/CD4/CD8 & other than <i>H. pylori</i>	60	2.4	142	19.3 [18.8;19.8]	0.47 [0.46;0.48]
- Other than <i>H. pylori</i> /CD3/CD4/CD8	244	2.0	484	65.9 [64.2;67.5]	1.63 [1.59;1.67]
- Oncologic	196	3.4	670	91.2 [88.9;93.4]	2.40 [2.34;2.46]

For **non-oncological indications**, the main stains used in GI biopsies are *H. pylori* (52.6% of cases, when including *H. pylori* staining carried out in combination with other stains, and 37.9% counting only the cases in which *H. pylori* was the only IHC stain performed), CD3 (8.4%), P53 (6,1%), CMV (4,7%), while the most frequently used in clearly **oncologic indications** appear to be MSI (12,1% of all oncologic GI IHC stains), CK-BS (8,3%), CDX2 (5,6%), CK20 (5,2%), P53 (5,1%) and CK7 (4,6%).

In 2019, there was high variability in the use of *H. pylori* staining across the 67 Belgian laboratories performing such tests (Figure 6). The median number of *H. pylori* stains was 2.4 x10³ (IQR :1.1 x10³ ; 3.7 x10³), which corresponds to a median of 23.0 % [IQR: 14.3 % ; 33.0 %] of all IHC stains and 56.9 % [IQR: 46.8 % ; 76.2 %] of GI stains. The median number of isolated *H. pylori* stains was 1.5 x10³ (IQR :0.6 x10³ ; 2.8 x10³), representing a median of 15.2 % [IQR: 6.0 % ; 28.6 %] of all IHC stains and 40.0 % [IQR: 24.2 % ; 66.9 %] of GI stains. Finally, there were three laboratories that did not conduct *H. pylori* IHC staining.



Figure 6 – Number of *H. pylori* stains per laboratory in 2019 (source: NIHDI)





2.2.5 Prescribers of IHC

In the sample of IHC for the year 2019 drawn by the NIHDI, a link was made between the number of IHC stains per body site and the prescribers. Table 9 offers an overview of the results.

Prescribers are registered by the NIHDI number and classified according to their field of specialisation.^f

Over the period 2012-2021, gastro-enterologists were the most frequent prescribers of diagnostic IHC tests (i.e. : 588070, 588081).

Data show that the proportion of prescriptions is decreasing for pathologists and internal medicine specialists, while it is increasing for dermatologists. Two pathologists were consulted about their prescribing role and the reason why the proportion of IHC prescribed by them is so limited. This consultation highlighted the fact that prescribing by pathologists is limited to second readings for cervical cytology in Belgium (nomenclature code: 588873).

Table 9 – Number of IHC stains (x10³) per category of prescriber (source: EPS)

Prescriber	2012	2014	2016	2017	2018	2019	2020	2021	Proportion Total
Gastro-enterologists	208.3	246.6	309.3	331.2	338.9	387.8	341.6	420.1	30.8%
Gynaecologists	81.6	95.5	124.4	128.2	128.9	149.3	146.6	175.5	12.3%
Dermatologists	68.8	75.4	93.6	105.7	125.5	133.9	152.6	184.8	11.0%
Surgeons	65.9	77.4	98.2	89.6	94.0	108.6	101.1	103.8	8.9%
Specialists in Radio-diagnostic	45.5	50.0	64.1	74.6	77.7	87.7	90.6	96.2	7.0%
Urologists	47.0	49.4	59.2	63.4	70.9	73.5	67.9	86.4	6.2%
Specialists in internal medicine	40.2	45.5	54.2	57.6	58.3	58.1	57.7	54.5	5.3%
Pneumologists	25.0	32.4	34.6	38.6	45.2	45.6	38.8	38.6	3.6%
Pathologists	40.1	48.4	26.5	28.5	27.2	31.4	26.4	28.8	3.3%
Plastic Surgeons	15.0	18.8	22.9	25.0	26.9	32.9	34.0	33.0	2.5%
ENT specialists	12.7	18.1	19.0	20.6	23.8	23.1	24.5	23	2.0%
Neuro-surgeons	10.6	12.5	15.9	15.1	15.1	16.8	13.1	14.9	1.4%
Paediatricians	7.4	10.2	11.4	11.2	11.0	13.2	8.9	11.3	1.0%
GPs	4.5	6.2	7.2	9.4	9.5	10.0	10.2	9.6	0.8%
Oncologists	8.1	7.0	7.2	8.3	8.4	8.7	8.1	7.9	0.8%
Orthopaedists	5.5	5.9	6.2	7.9	9.4	10	7.0	8.0	0.7%

^f https://www.inami.fgov.be/SiteCollectionDocuments/codes_competences_medecins.pdf



Stomatologists	3.0	5.1	6.7	5.0	9.0	8.0	8.6	8.9	0.7%
Cardiologists	6.1	6.7	6.9	5.3	6.8	6.4	4.6	5.3	0.6%
Other	10.4	9.5	12.4	11.7	10.7	13.6	13.7	13.0	1.2%
Total	705.8	820.6	980.0	1036.7	1096.9	1218.5	1156.1	1323.7	100%

Other prescribers included anaesthetists, cardiologists, orthopaedists, GP, emergency specialists, neurosurgeon, ENT, oncologists, paediatricians, stomatologists.

2.2.6 Trends in procedures

The following procedures were studied: GI and gynaecological procedures, skin biopsies, prostate biopsies, breast sampling (i.e biopsies and fine needle aspiration) and general surgical procedures, as these were identified as the most common procedures linked to the most frequent biopsy sites in the pathology reports data received from the NIHD and previously described (see section 2.2.4.1 for more details). When looking at these, data show that gastroscopies represent 60% of all GI procedures.

GI and skin biopsies, as well as breast sampling have all increased over the study period. However, for GI, the increase appears to be associated with a higher number of colonoscopies, while the number of gastroscopies has decreased. The steep increase in colonoscopies is likely to be a consequence of the recent public efforts to increase the rates of colorectal cancer detection.⁹ An important reduction of gynaecological procedures was also seen over the studied period. Such decrease may be due to changes in reimbursement of pap smear tests (from 1 every 2 years, to 1 every 3 calendar years) that took place in Belgium in March 2013. See Table 10 for more information.

Table 10 – Evolution in number of procedures (x10³) 2012-2021 (source: Doc N)

	2012	2014	2016	2017	2018	2019	2020	2021	% increase (2012-2019 ?)
Gastro-intestinal	645.0	687.3	704.5	767	745.7	749.2	628.3	736.9	16.2%
- Gastroscopy	435.8	431.9	437.1	429.7	410.9	405.4	339.1	394.9	-7.0%
- Colonoscopy	209.3	255.4	267.5	337.3	334.8	343.9	289.2	342	64.3%
Skin biopsy	175.2	183.7	191.9	193.8	199.9	203.4	186.4	213.8	16.1%
Breast sampling	51.1	52.6	51.5	51.6	52.8	53.2	51.3	58.5	4.1%
Gynaecology	343.4	160.0	164.2	159.7	157.2	163.8	145.3	166.3	-52.3%
Prostate biopsy	13.2	11.6	13.0	13.4	13.9	13.6	13.1	15.7	2.9%
Surgery	116.2	119.5	121.2	118.5	122.0	127.3	105.4	123.5	9.5%

⁹

<https://www.ccref.org/pro/#/>;
<https://www.bruprev.be/fr/bruprev>

<https://bevolkingsonderzoek.be/nl/>



The research team looked at the number of IHC stains linked to each of the studied procedures. Globally, the number of procedures after which (at least) one IHC stain was performed, increased over the period 2012-2021. Moreover, the mean number of IHC stains per procedure also increased for all the selected procedures. Both parameters explain the general growth in the total number of IHC stains.

Overall, in 2019, 85% of all IHC stains are attributed to gastroscopies for the purpose of our analysis. However, this may be an overestimation, since GI biopsies included both colonoscopies and gastroscopies, two procedures

which were performed at the same time (same contact/session) in 20% of the cases. It is important to highlight that the proportion of cases in which these two procedures have been performed together increased during the study period (excluding the COVID pandemic years) from 12% in 2012 to 20% in 2019.

Table 11, summarises the number of procedures for which IHC staining was performed, the number of IHC stains and the mean number of stains per sampling.

Table 11 – Number of IHC stains (x10³) according to medical procedure (source: EPS)

		2012	2014	2016	2017	2018	2019	2020	2021
Gastro-intestinal	N. of procedures with IHC (x10 ³)	152.5	171.0	202.3	216.7	215.6	234.5	204.4	245.5
	Percentage with histology	99.8%	99.8%	99.8%	99.8%	99.9%	99.8%	99.4%	99.6%
	Total N. of IHC stains (x10 ³)	259.4	297.8	367.9	397.8	400.7	453.1	401.9	473.4
	Mean N. IHC/sampling	1.70 ± 0.02	1.74 ± 0.02	1.82 ± 0.02	1.84 ± 0.02	1.86 ± 0.02	1.93 ± 0.02	1.97 ± 0.02	1.93 ± 0.02
Skin biopsy	N. of procedures with IHC (x10 ³)	22.9	25.0	31.2	36.2	40.1	44.3	44.4	53.0
	Percentage with histology	97.1%	99.3%	98.9%	99.1%	98.8%	99.1%	99.2%	99.4%
	Total N. of IHC stains (x10 ³)	72.1	79.7	97.8	110.6	124.7	136.1	145.7	171.0
	Mean N. IHC/sampling	3.15 ± 0.07	3.19 ± 0.07	3.14 ± 0.06	3.06 ± 0.05	3.11 ± 0.05	3.07 ± 0.05	3.28 ± 0.05	3.23 ± 0.04
Breast sampling	N. of procedures with IHC (x10 ³)	17.4	18.2	18.6	20.5	21.3	25.4	23.2	24.9
	Percentage with histology	99.4%	99.7%	99.9%	99.6%	99.8%	99.8%	99.5%	99.8%
	Total N. of IHC stains (x10 ³)	70.2	76.4	86.0	91.7	97.5	115.1	112.2	115.5
	Mean N. IHC/sampling	4.03 ± 0.1	4.19 ± 0.1	4.63 ± 0.11	4.48 ± 0.1	4.58 ± 0.1	4.54 ± 0.09	4.83 ± 0.1	4.64 ± 0.09
Gynaecology	N. of procedures with IHC (x10 ³)	18.6	18.7	24.1	24.9	25.0	28.2	26.6	33.5
	Percentage with histology	99.9%	99.4%	99.5%	99.8%	99.7%	99.7%	99.9%	99.9%
	Total N. of IHC stains (x10 ³)	49.6	50.1	71.9	73.5	73.9	81.9	82.0	101.0



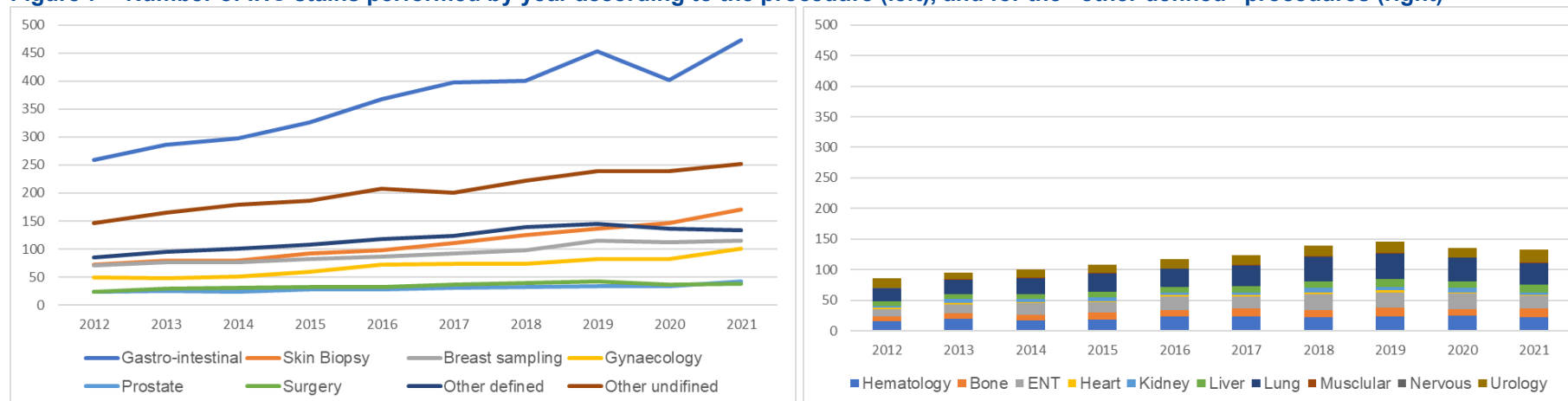
	Mean N. IHC/sampling	2.67 ± 0.07	2.68 ± 0.07	2.99 ± 0.07	2.95 ± 0.06	2.96 ± 0.06	2.90 ± 0.06	3.08 ± 0.06	3.01 ± 0.06
Prostate biopsy	N. of procedures with IHC (x10 ³)	7.3	7.0	7.8	8.3	9.1	9.9	9.6	12.3
	Percentage with histology	99.5%	100,00%	100,00%	100,00%	99.6%	100,00%	99.6%	99.8%
	Total N. of IHC stains (x10 ³)	23.6	23.4	27.6	30.2	32.5	33.5	33.7	42.5
	Mean N. IHC/sampling	3.22 ± 0.08	3.33 ± 0.07	3.54 ± 0.09	3.64 ± 0.08	3.57 ± 0.08	3.37 ± 0.07	3.50 ± 0.08	3.46 ± 0.06
Surgery	N. of procedures with IHC (x10 ³)	8.2	10.7	11.4	12.0	13.8	13.9	11.6	12.4
	Percentage with histology	99.3%	99.4%	99.5%	98.8%	99.4%	99.7%	99.5%	99.7%
	Total N. of IHC stains (x10 ³)	24.0	30.5	32.0	36.2	39.2	42.9	37.0	37.5
	Mean N. IHC/sampling	2.93 ± 0.12	2.85 ± 0.09	2.81 ± 0.1	3.00 ± 0.11	2.85 ± 0.09	3.09 ± 0.09	3.18 ± 0.1	3.02 ± 0.09
Other	N. of procedures with IHC (x10 ³)	71.2	85.3	95.8	95.9	104.2	113.0	108.7	116.1
	Percentage with histology	96.9%	97.7%	98.1%	97.5%	97.8%	97.4%	97.4%	97.8%
	Total N. of IHC stains (x10 ³)	232.0	280.1	324.4	324.3	361.1	385.3	375	385.5
	Mean N. IHC/sampling	3.26 ± 0.04	3.28 ± 0.04	3.39 ± 0.04	3.38 ± 0.04	3.46 ± 0.04	3.41 ± 0.03	3.45 ± 0.03	3.32 ± 0.03

Selected nomenclature for the procedures: For Gastro-intestinal: 472356, 472360, 473056, 473060, 473093, 473104, 472452, 472463, 473174, 473185, 473432, 473443, 474751, 474762; For gynaecology: 431966, 431955, 432121, 432110, 432143, 432132, 432375, 432386, 432390, 432401, 432412, 432423, 432530, 432541, 432574, 432585, 432596, 432600, 432611, 432622, 432633, 432644, 432655, 432666, 432670, 432681, 432736, 432740; For skin: 532652, 532663, 532674, 532685, 532836, 532840, 532851, 532862, 532125, 532114, 532022, 532011; For prostate: 355832, 355843; For breast sampling: 355213, 355224, 355235, 355246, 355250, 355261, 227091, 227102, 227684, 227684, 355670 355681; For surgery: 220275, 220286, 220290, 220301, 220231, 220242, 220150, 220161, 220334, 220345, 220113, 220124, 220264, 220253, 220312, 220323, 220371, 220382, 221196, 221200, 220356, 220360, 221152, 221163, 221034, 221045, 220091, 220102, 221012, 221023, 220135, 220146, 220216, 220220, 221185, 221082.

Specific analyses on gastroscopies are presented in section 2.2.7. For the category labelled as "other", some procedures could be defined, allowing additional grouping (see Figure 7). The undefined "other" (for which a specific procedure could not be identified) included macro biopsies, (retro)-peritoneal punctures and lymph node punctures.



Figure 7 – Number of IHC stains performed by year according to the procedure (left), and for the “other defined” procedures (right)



Selected nomenclature for the procedures are: For haematology: 355714, 355725, 355736, 355740, 275030, 275041, 474596, 474600, 280210, 280221, 275015, 275026, 300252, 300252, 280195, 280195, 355412, 355423, 300274, 300285; For ENT: 351035, 351046, 258834, 258845, 258510, 258521, 311953, 311964, 255791, 255802, 311975, 311986, 257364, 257364, 258075, 258086, 256594, 256605; For heart: 476652, 476663, 355471, 355482; for kidney: 355795, 355806, 355810, 355821; For liver: 242373, 242384, 355751, 355762; For lung: 355655, 355666, 257320, 471741, 471811, 471822, 227452, 227463, 355633, 355644, 471052, 471063, 471855, 471866, 355434, 355445, 471752, 471763; For muscular: 280770, 280781; For bone: 355714, 355725, 355736, 355740, 275030, 275041, 474596, 474600, 280210, 280221, 275015, 275026, 300252, 300252, 280195, 280195, 355412, 355423, 300274, 300285; For urology: 260271, 260282, 260330, 260341, 261531, 261542, 431152, 431163, 355854, 355865, 461635, 461646.

While the previously studied NIHD data, highlighted “lung” as the 5th most frequent body site for biopsies in which IHC was performed, in the EPS data here explored, “lung” seems to represent only a small proportion of all IHC stains. This can be explained by the definition of the indication. In the NIHD data, the indications are determined by the information captured in the pathology reports. In the EPS data, the indications are determined by the procedures performed in the 3 months before the invoice for the IHC stain, and thus, more than one procedure can be performed for the same body site. In the case of lung, both surgery, or pulmonary sampling can be attributed to lung.



Conclusion – Indications and trends (per indication) for IHC testing in Belgium

- The main biopsy sites in which IHC stains are performed in Belgium are GI, followed by breast, skin and cervix.
- For non-oncological indications, *H. pylori* staining was performed in 52.6% of GI biopsies during the year 2019, (and in 37,9% of such biopsies, it was the only IHC stain performed).
- In Belgium for the last 10 years, gastro-enterologists are the most frequent prescribers of IHC tests (approximately 30.8%). The proportion of prescriptions by pathologists is very low (around 3.3%).
- Different procedures were studied: GI, gynaecology, skin biopsies, prostate biopsies, breast sampling and general surgery procedures. GI biopsies included colonoscopies and gastroscopies (the latter accounted for 60% of all GI biopsies in 2019).
- Our data show a global increase in the use of IHC stains for all the selected procedures, as a result of:
 - **A general increase in the number of procedures: the number of gastro-intestinal, skin biopsies, and breast sampling all increased over the study period. However, for GI, the increase appears to only be associated to a higher number of colonoscopies, while the number of gastroscopies decreased.**

- **An increase on the number of procedures on which at least one IHC was performed.**
- **An increase in the mean number of IHC stains per procedure for all the selected procedures.**

2.2.7 A zoom in on Gastroscopies

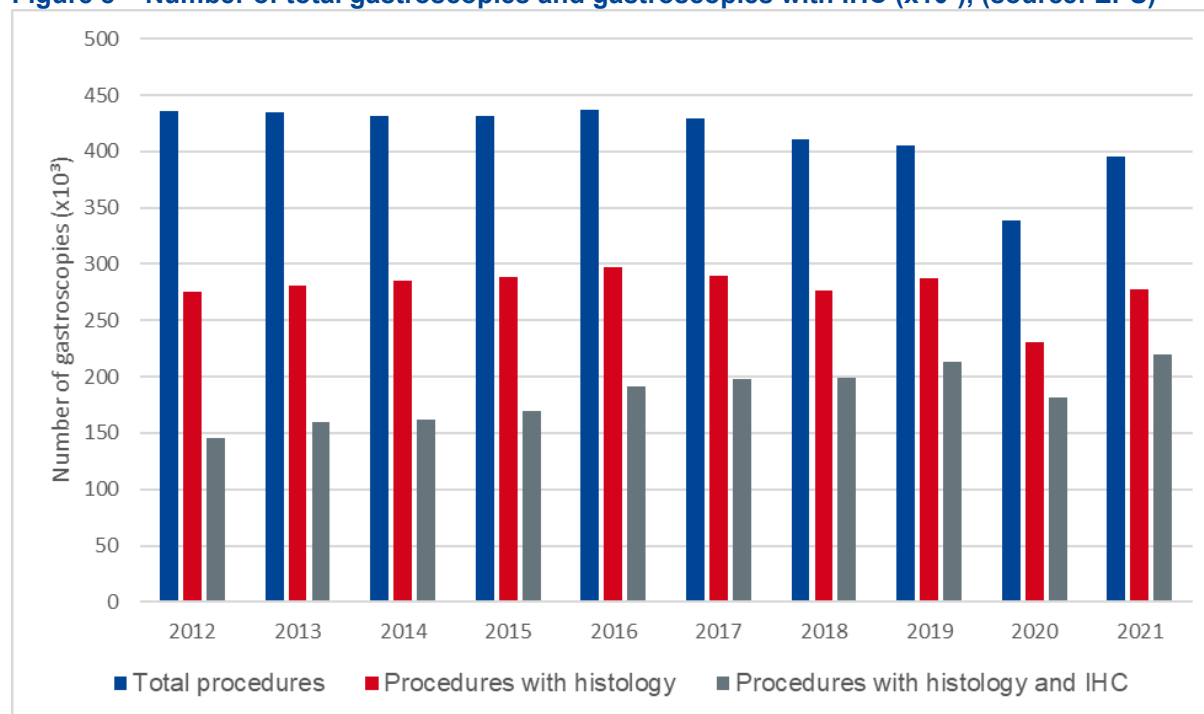
2.2.7.1 Number of procedures

Figure 8 shows the total number of gastroscopies, the number of gastroscopies for which a histology analysis has been performed and the number of gastroscopies for which histology and IHC staining have been performed.

The number of procedures and histology appear to have remained relatively stable over the period 2012-2019, excluding the COVID pandemic years, (-7.0 and +4.3% respectively). However, the number of procedures on which IHC has been performed increased over that same period (+ 46.1% from 2012-2019,).



Figure 8 – Number of total gastroscopies and gastroscopies with IHC ($\times 10^3$), (source: EPS)





2.2.7.2 Evolution by type of setting

The number of procedures performed decreased over the years 2012-2019 (excluding the COVID pandemic period) for general hospitals with a university character and for the out of hospital setting, while it increased in university and general hospitals. In all cases, the use of IHC staining increased.

Table 12 – Gastroscopies: Number of total procedures and procedures with IHC staining (x10³), (source: EPS)

Institution type		2012	2014	2016	2017	2018	2019	2020	2021	% increase (2012-2019)
University	Total N. of procedures	40.0	36.7	40.7	40.0	36.2	42.3	34.4	37.1	5.6%
	Procedures with histology	26.3	23.9	28.1	26.5	23.4	27.5	21.3	23.5	4.6%
	Procedures with histology and IHC	16.3	15.3	18.2	16.7	16.4	19.0	16.0	17.7	16.2%
General hospitals with a university character	Total N. procedures	74.8	79.8	79.0	78.3	73.7	73.1	54.4	70.3	-2.3%
	Procedures with histology	45.3	51.1	53.8	53.0	50.4	49.8	35.1	46.4	10.1%
	Procedures with histology and IHC	27.6	34.0	40.9	40.1	38.9	38.9	27.4	36.0	41.0%
General hospitals	Total N. procedures	238.7	238.6	251.3	247.1	234.3	243.1	199.8	235.8	1.8%
	Procedures with histology	156.3	160.9	173.2	169.3	166.7	174.0	145.1	172.3	11.4%
	Procedures with histology and IHC	79.6	88.0	108.9	115.9	120.9	130.9	117.1	139.7	64.5%
Out of hospitals	Total N. procedures	82.5	77.2	66.3	65.6	56.3	54.5	43.7	49.9	-33.9%
	Procedures with histology	47.3	49.4	41.5	40.8	36.1	35.5	29.3	34.8	-24.8%
	Procedures with histology and IHC	22.4	24.0	23.6	25.2	23.2	24.4	20.8	26.2	9.0%



2.2.7.3 Trends in the N. of gastroscopies per age group

An additional analysis was performed on gastroscopies performed during the same period, by age group.

Table 9 – Number of gastroscopies (x10³), associated histology and IHC by age category (Source:EPS)

	2012	2014	2016	2017	2018	2019	2020	2021	% increase (2012-2019)
Age <18 Gastroscopies	10.3	10.3	11.2	9.6	8.8	10.3	7.6	9.8	0.0%
- With Histology	8.3	8.3	9.7	8.0	7.3	8.6	6.2	7.9	3.6%
- With Histology and IHC	5.2	5.9	7.1	6.4	5.9	6.7	5.4	6.7	28.8%
Age [18-49] Gastroscopies	140.8	135.6	135.2	134.8	124.3	126.7	100.9	120.6	-10.0%
- With Histology	97.3	99.5	101.4	100.8	95.8	97.8	77.5	94.8	0.5%
- With Histology and IHC	50.6	57.3	66.2	68.6	70.3	73.7	62.1	75.4	45.7%
Age ≥50 Gastroscopies	285.0	286.4	290.8	286.6	267.3	276.0	223.8	262.6	-3.2%
- With Histology	169.5	177.4	185.6	180.9	173.4	180.5	147	174.4	6.5%
- With Histology and IHC	90.1	98.2	118.3	122.9	123.2	132.8	113.8	137.5	47.4%

In the group aged <18, almost all gastroscopies are followed by a pathological investigation, compared with the other age categories, where this is not always the case. For this age category, although the number of gastroscopies remained stable during the study period, the number of IHC performed increased by 28.8%. For the age category 18-49 years, the number of gastroscopies decreased by 10%, from 140.8 in 2012 to 126.7 in 2019, and for the age category ≥50 years by 3.2%, from 285.0 in 2012 to 276.0 in 2019. For each of these two categories of age, the number of IHC performed increased by approximately 46% from 2012 to 2019.

2.2.7.4 Prescribers

The number of pathological tests performed on gastroscopies increased by 19% in the period 2012-2019 (Table 13), but while the growth in the number of standard histology (H&E coloration) was 7.6%, the increase in IHC staining was greater. This trend is a consequence of both an increase in the number of histology in which at least one IHC stain was performed (+46.1%), and an increase in the mean number of IHC stains performed per biopsy (+12.5%).

Gastro-enterologists were the most common prescribers (in 2019, 82% of all reimbursed tests).

**Table 13 – Number of anatomopathological, histology and IHC stains (x10³) performed on gastroscopies in 2012-2021 (Source: EPS)**

	2012	2014	2016	2017	2018	2019	2020	2021	% increase (2012-2019)
Total number of pathological tests	902.2	959.2	1056.0	1040.2	1014.5	1073.7	890.2	1089.6	19.0%
Total number of histology (% of the total number of pathological tests)	350.6 (38.9%)	368.6 (38.4%)	387.9 (36.7%)	373.6 (35.9%)	359.1 (35.4%)	377.3 (35.1%)	302.6 (34.0%)	366.3 (33.6%)	7.6%
Total number of histology with min one IHC stain	145.9	161.4	191.6	197.9	199.4	213.2	181.3	219.6	46.1%
Average number of IHC stains per biopsy	1,7	1,7	1,8	1,8	1,8	1,9	1,9	1,9	12.5%

Conclusion – Gastroscopies in Belgium

- More than 400 000 gastroscopies were performed each year from 2012 to 2019. This number tended to be stable until 2020 where a decrease was observed. In 2021, 393 000 gastroscopies were carried out.
- Histology was performed in 65 to 70% of gastroscopies (i.e. proportion of gastroscopies in which a biopsy was taken). IHC staining was carried out in 53% of these biopsies in 2012 and on 79.2% in 2019, which represents an increase of 46%.
- IHC staining grew in all setting types despite the fact that the number of gastroscopies performed decreased over the studied years, both in general hospitals with a university character and in the out-of-hospital setting.
- For the group aged <18, almost all gastroscopies are followed by a pathological procedure, compared with the other age categories, where it is less common.
- The growth in IHC testing over the last decade was higher than the growth in pathological testing in general.

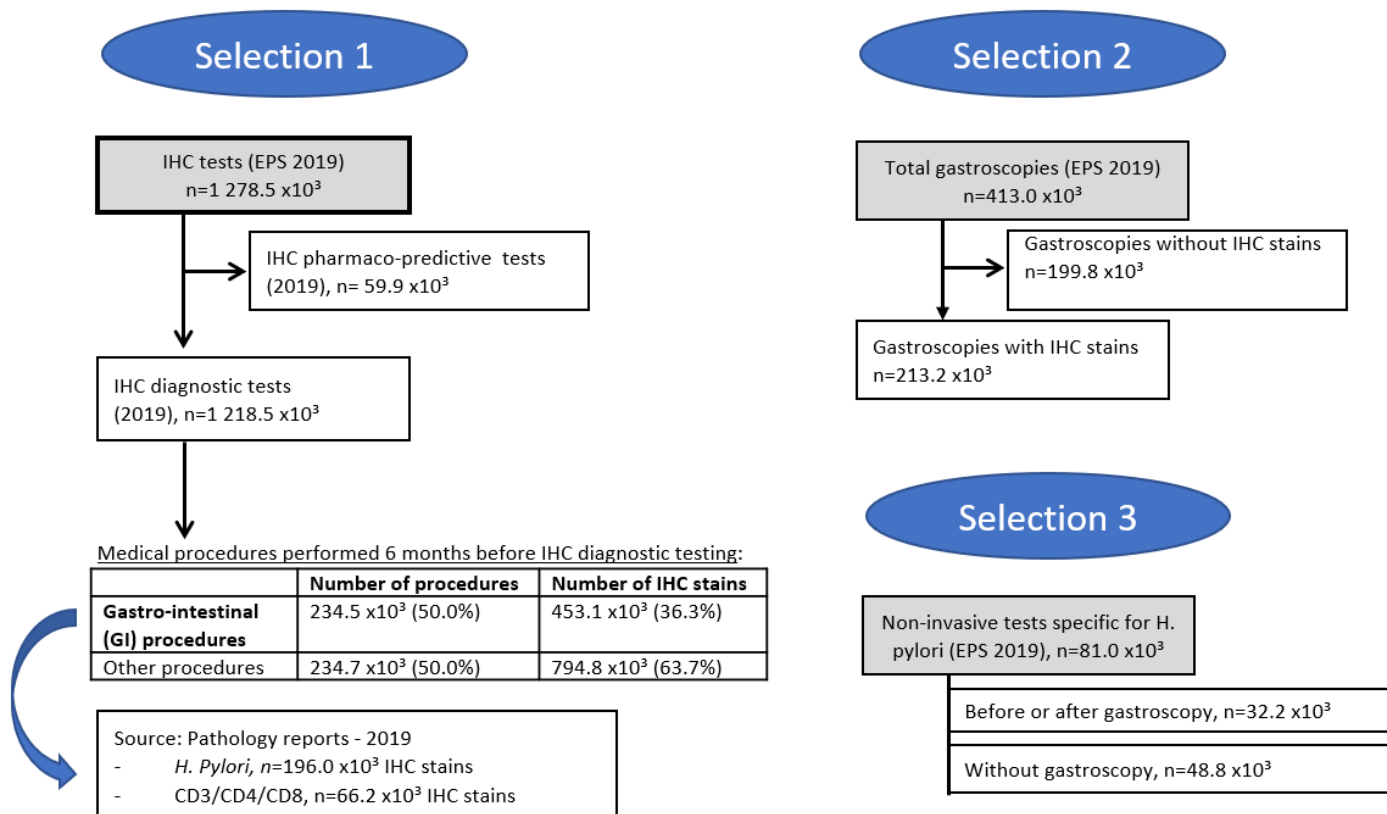


2.2.8 Case studies for *H. pylori* and coeliac disease diagnoses in Belgium

For the purpose of these case studies, the data from the EPS had to be combined with data extracted by the NIHDI from pathology reports, which are only available for the year 2019. Therefore, most of the analysis is limited to that year and no exploration of trends was possible.

Figure 9, shows the flow of the data and the number of cases and tests that were considered.

Figure 9 – Flow chart of the selection of data (multiple sources) for the year 2019 identifying *H. pylori* cases.





2.2.8.1 A case study - diagnosis of *H. pylori* in Belgium

The first case study focuses on *H. pylori*, given the importance this indication has for IHC use. Our analysis showed that *H. pylori* is the most common non-oncologic diagnostic indication for IHC.

Its detection can be facilitated using both invasive (biopsy on gastroscopies) or non-invasive tests.

Non-invasive tests include:

- Serology, most often done before a gastroscopy, to detect primary antibodies. This test is no longer reimbursed in Belgium since 01/01/2021.
- Stool Ag tests.
- Urea Breath Tests: (UBT), most commonly performed after treatment to control the eradication of *H. pylori*.

The presence of a positive *H. pylori* test is not captured as such in any of the consulted sources and was thus, defined as either the registration of *H. pylori* treatment, according to Belgian recommendations.⁸ or by the identification of an *H. pylori* eradication test (UBT) in the 3 months post initial *H. pylori* test (invasive or non-invasive).

A full list of the specific therapies recommended and considered in this analysis is offered in an appendix and can be summarized as:

- A combination of at least 2 antibiotics among the following: Tetracycline, Clarithromycin, Amoxicillin or Metronidazole during minimum 10 days.
- Used in combination with PPIs;

The main combination of antibiotics are detailed in Table 14. The most common was Clarithromycin/Amoxicillin (85.4%), followed by Metronidazole/Amoxicillin (7.6%) and Tetracycline/Amoxicillin (5.5%). Rarely, the combination Clarithromycin/Metronidazole (0.4%), or a combination of 3 antibiotics (<1%) are used.

Despite the recommendation on the possible use of Bismuth or the three-in-one capsule containing bismuth, tetracycline and metronidazole (i.e. Tryplera), and the confirmation by experts that these therapies are used in Belgium, they are not currently reimbursed^h, and are therefore, not captured in the administrative database explored for the purpose of this analysis. This should be recognized as a further limitation of the available data (underestimation of positive cases).

Table 14 – Association of antibiotics used in the treatment of *H. Pylori* (Source: EPS)

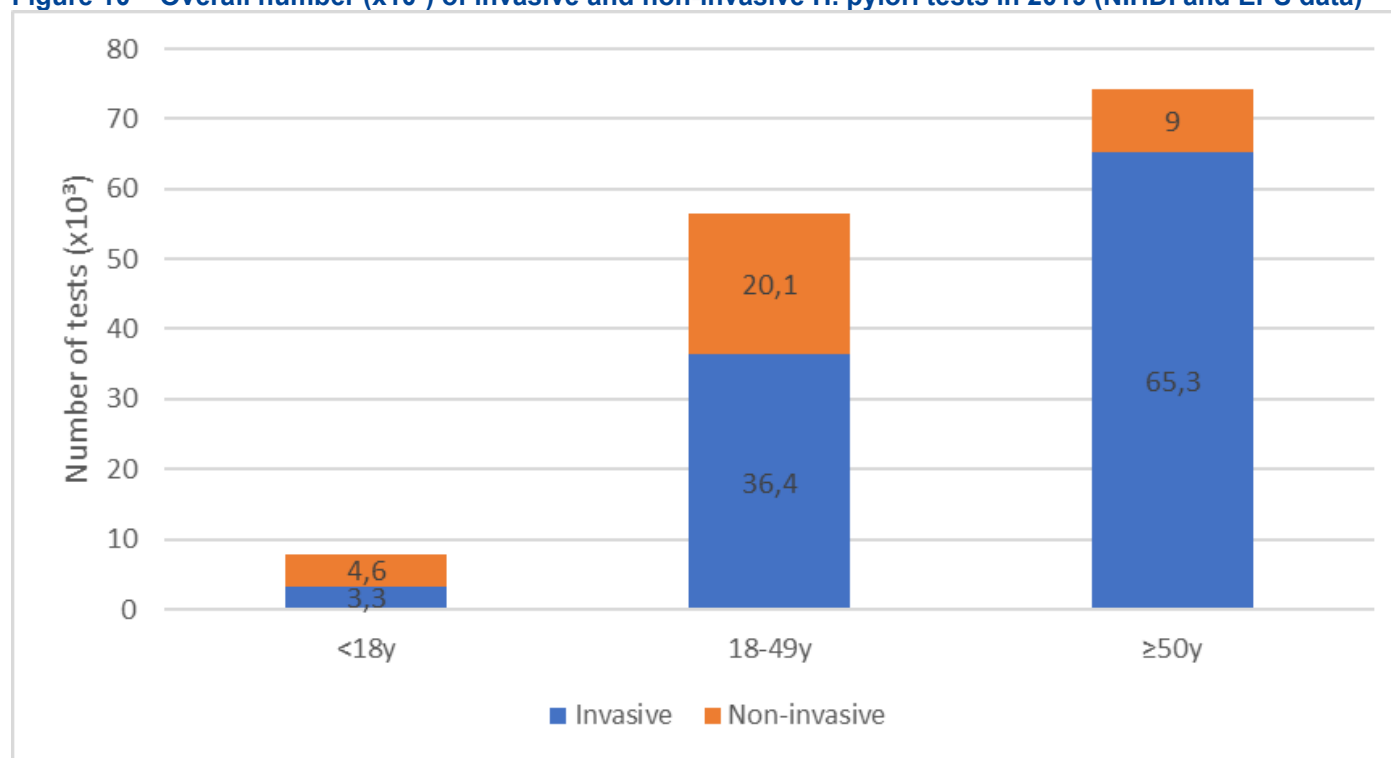
Clarithromycin	Metronidazole	Amoxicillin	Tetracycline	% of combination
x		x		85.4%
	x	x		7.6%
		x	x	5.5%
x		x	x	0.4%
x	x			0.4%
x	x	x		0.3%
	x	x	x	0.2%
	x		x	0.1%
x			x	0.1%

^h Medication registered in the NIHD Category D for which no reimbursement is foreseen.



2.2.8.2 Invasive (gastrosopies) and non-invasive *H. pylori* tests

Figure 10 – Overall number ($\times 10^3$) of invasive and non-invasive *H. pylori* tests in 2019 (NIHDI and EPS data)



For invasive tests, as previously estimated based on the NIHDI data derived from the pathology reports and captured in Table 8, 196.0×10^3 [191.1×10^3 ; 200.9×10^3] IHC stains were performed for *H. pylori* in 2019.

Data from the EPS, show that on that same year, 413.0×10^3 gastroscopies were carried out, on which a total of 213.2×10^3 IHC stains with histology were performed. The distribution by age category allows us to extrapolate the number of IHC stains performed specifically for *H. pylori*.

It is important to mention that *H. pylori* testing by invasive methods can be performed for other reasons than the diagnoses of gastrointestinal complaints. *H. pylori* culture and antibiotic sensitivity testing is an example (e.g. clarithromycin susceptibility testing, if available through molecular techniques or culture, is recommended before prescribing any clarithromycin containing therapy⁹). In Belgium, 1.7×10^3 *H. pylori* culture and antibiotic sensitivity tests were performed in 2019. A further example is the *H. pylori* culture routinely performed in patients undergoing bariatric surgery.



In our 2019 studied population, 9.9×10^3 patients underwent bariatric surgery after a gastroscopy. This represents 81.4% of all the bariatric surgeries performed on that year. **For non-invasive tests**, in order to offer an insight into the use of such tests for *H. pylori*, it is important to differentiate those tests carried out in patients who had also undergone an invasive test (gastroscopy) and those performed in the absence of any invasive testing.

Given our focus on diagnoses, it is also important to separate non-invasive tests undertaken after a gastroscopy (within an 3-month period), which are likely to represent eradication tests, from those performed prior to the gastroscopy, assumed to be *H. pylori* diagnostic tests.

Data for the year 2019 shows that 9.0% of serologies, 9.2% of the stool antigen tests and 8.7% of the UBT are carried out before a gastroscopy and can therefore be assumed to represent diagnostic *H. pylori* tests.

Our data show that the main prescribers of non-invasive tests in Belgium are GPs (57% of all non-invasive test prescribers).

Table 15, shows that in 2019 the number of non-invasive tests for *H. Pylori* represents one fifth of the number of invasive tests for *H. Pylori*. About 40% of the non-invasive tests for *H. Pylori* are performed before or after gastroscopy, including UBT tests done after a gastroscopy, (i.e. eradication tests), which represent 55% of these tests.

Table 15 – Invasive (gastroscopies) and non-invasive tests ($\times 10^3$) for *H. pylori* diagnosis in 2019, by age category (Sources: NIHDI data from pathology reports and EPS)

Age (years)	<18	18-49	≥50	Total
INVASIVE TESTS				
Number of gastroscopies for <i>H. Pylori</i>	3.3	36.3	65.3	105.0
Number of IHC stains for <i>H. Pylori</i>	6.5	59.4	130.2	196.0
NON-INVASIVE TESTS PERFORMED BEFORE AN INVASIVE TEST (max 3 months)				
Serology	0.3	1.2	0.8	2.4
Stool Ag test	0.08	1.5	0.4	1.9
UBT	0.4	1.1	1.4	2.9
NON-INVASIVE TESTS PERFORMED AFTER AN INVASIVE TEST (max 6 months)				
Serology	0.08	1.4	1.3	2.7
Stool Ag test	0.1	2.2	1.5	3.9
UBT	0.7	8.4	9.3	18.4
NON-INVASIVE TESTS PERFORMED WITHOUT INVASIVE TEST				
Serology	3.0	10.6	7.6	21.2
Stool Ag test	1.1	12.4	1.9	15.4
UBT	1.9	5.9	4.4	12.2



2.2.8.3 Positive *H. pylori* tests

For the invasive tests, a proxy for a positive *H. pylori* test result was estimated by considering either a *H. pylori* pharmacological treatment in the 6 months before the test or in the 3 months after the test, or the presence of a non-invasive UBT test 3 to 12 months after gastroscopy, or a second gastroscopy performed more than 30-days and maximum one year after the initial one. It is important to highlight that there could be different reasons to perform a further gastroscopy (within three months) such as carrying out an *H. pylori* culture and antibiogram when eradication failure has been observed, checking for dysplasia in a context of extensive gastric metaplasia after sedation of inflammatory compound, or checking for dysplasia in a Barrett oesophagus after sedation of inflammation.

For the non-invasive tests without gastroscopy, a proxy for a positive result was estimated by considering either a *H. pylori* pharmacological treatment in the 6 months before the test, or a UBT test during in the 3 to 12 months after the initial non-invasive test.

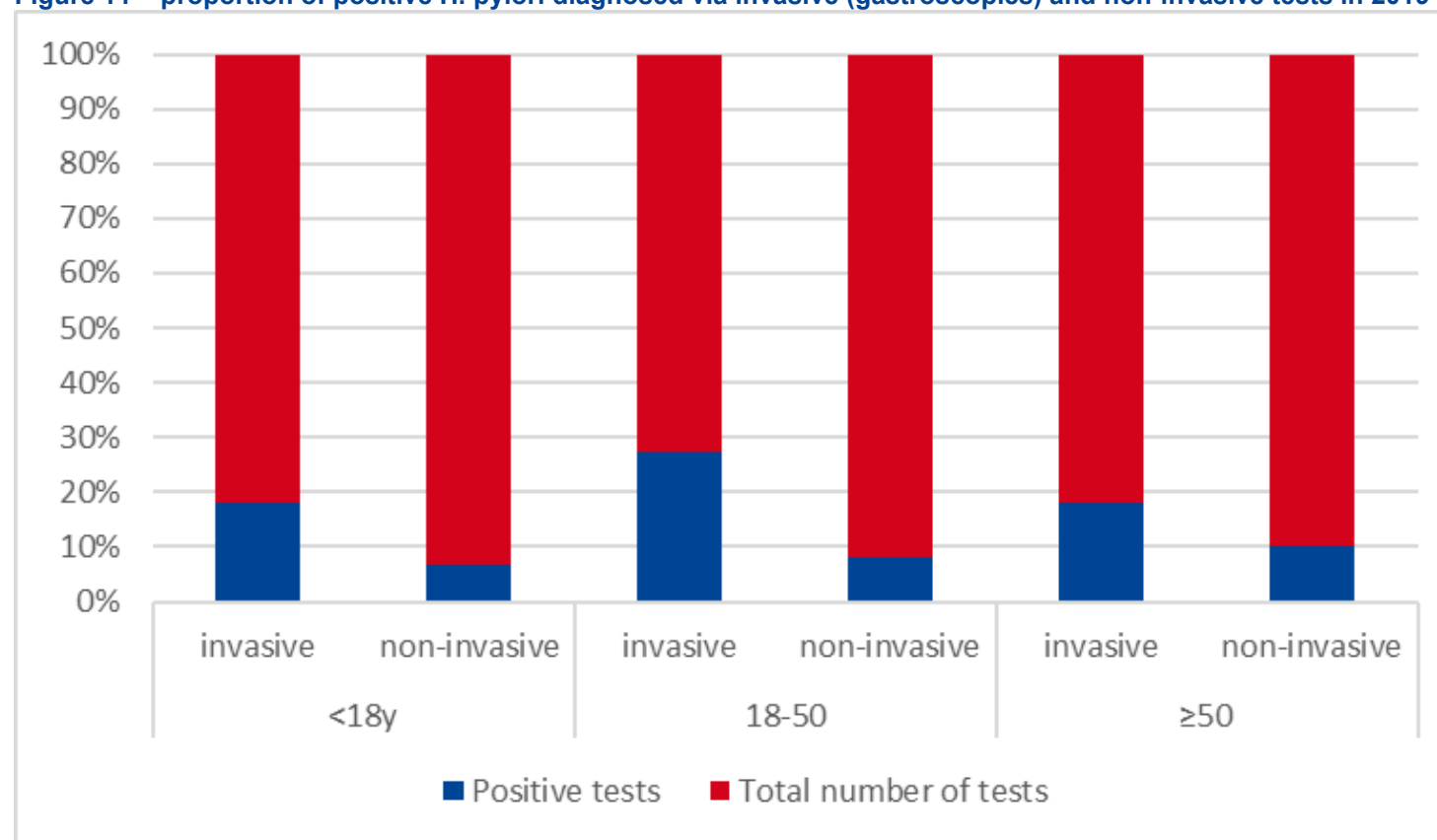
Table 16 shows the number of contacts meeting the previously described criteria for (proxy) positive *H. pylori* testing in 2019. For invasive tests 22.2 x10³ contacts were considered positive (21.1%), while for non-invasive tests, there were 3.7 x10³ identified as positive contacts (7.6%). Figure 11 shows the proportion of positive *H. pylori* tests via invasive and non-invasive techniques by age group.

Table 16 – Number of contacts (x10³) with positive *H. Pylori* tests in 2019

	Age (years)	<18	18-49	≥50	Total
INVASIVE TESTS					
- Number treated (6 months before or 3 months after)		0.2	6.9	7.7	14.8
- Number UBT tests during the 3 to 12 months after a gastroscopy		0.6	7.6	8.9	17.0
- Number of 2nd gastroscopies (>30days)		0.04	0.4	0.1	0.54
- Number of <i>H. pylori</i> culture and antibiogram after the 2nd gastroscopy		-	0.2	0.04	0.24
Number of positive invasive tests (combination of first 3 rows)		0.6 (2.7%)	9.9 (44.6%)	11.7 (53%)	22.2
NON-INVASIVE TESTS					
- Number treated (6 months before or 3 months after)		0.4	2.2	1.3	3.4
- Number UBT tests during the 3 to 12 months after the initial non-invasive test		0	0.2	0.1	0.3
Number of positive non-invasive tests (combination of the 2 previous rows)		0.4 (10.8%)	2.3 (62.0%)	1.4 (37.8%)	3.7

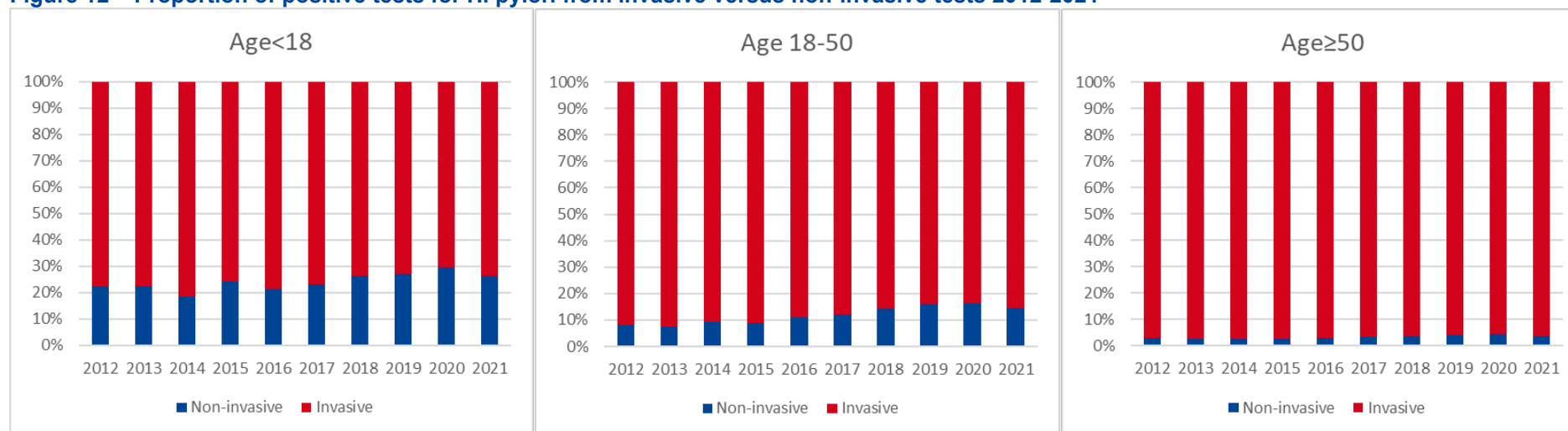


Figure 11 – proportion of positive *H. pylori* diagnosed via invasive (gastroscopies) and non-invasive tests in 2019



In the EPS data, treatment for *H. pylori* as well as eradication tests (i.e. monitoring successful eradication after treatment) were identifiable. This made it possible to look at the evolution, over the study period (2012-2021), of the total number of positive tests (invasive + non-invasive) by age group. Figure 12 shows the results. For the group aged <18, the positive tests via

non-invasive testing methods increased from 20% in 2012 to 30% in 2021. In the group aged 18-49, the positive tests via non-invasive testing also increased by 10% over that same period, reaching 15% in 2021. In the older group, positive tests were mainly detected by invasive methods.

**Figure 12 – Proportion of positive tests for *H. pylori* from invasive versus non-invasive tests 2012-2021****Conclusion – Diagnosis of *H. pylori* in Belgium**

- In 2019, *H. pylori* was the most common non-oncologic diagnostic indication for IHC in Belgium.
- Detection of *H. pylori* is possible either via non-invasive or invasive tests. The proportion of non-invasive testing in 2019 in Belgium was 58% in patients aged <18, 36% in patients aged 18-49 and 12% in patients aged ≥50.

- Positive *H. pylori* cases are mostly diagnosed by invasive testing, for all age categories. This is likely to be a consequence of both the higher use of invasive testing in general, and a difference in the profile of patients which are diagnosed by one type of test, or the other.
- The proportion of positive *H. pylori* cases diagnosed by non-invasive testing increased in the last 10 years (2012-2021) for all age groups, and reached in 2019 27% in the group aged <18, and 16% for patients aged 18-49, while it remained low (4%) in those aged ≥50.



2.2.9 A case study - diagnosis of Coeliac disease

The second case study focuses on coeliac disease, since our analysis of 2019 Belgian reimbursement data showed CD3, frequently used for the diagnosis for this disease, to be the second most common IHC stain in Belgium.

The detection of coeliac disease is based on a combination of clinical, serological and histopathological data. It initially involves a serologic test aimed at identifying the presence or absence of specific antibodies related to this disease. The most commonly recommended blood test is the Tissue Transglutaminase IgA antibody (TTG-IgA), in combination with an IgA antibody in order to ensure that the patient generates enough of this antibody to render the coeliac disease test accurate. For the results of these serologic tests to be valid, the individuals undergoing testing need to be on a gluten containing diet.^{10, 11}

In a second step, those patients in which the presence of the specific antibodies for coeliac diseases are detected via a serologic test, need to undergo an endoscopy in order to confirm the diagnosis. Recently published European guidelines for the paediatric population have established recommendations on some specific cases in which a biopsy may not be necessary for children.¹² The option, under certain very concrete circumstances, to base diagnosis purely on non-invasive methods and avoid endoscopy has also started to be discussed for the adult population. However, as we will see in our chapter on the review of the literature (see Section 3.3), current clinical guidelines still recommend adults in whom specific antibodies for coeliac diseases are detected by means of a serologic test, to always undergo an endoscopy.

Genetic testing can also be performed in this field to determine if a person carries the HLA-DQ2 or HLA-DQ8 gene. These are genes associated with an increased risk of coeliac disease. However, a positive genetic test cannot be used in isolation to diagnose this disease. Instead, it can be helpful for ruling it out if the genes are not present.

The only existing treatment consists on following a strict, life-long gluten free diet.

2.2.9.1 Non-invasive testing: serology to detect anti-deaminated gliadin antibodies, anti-endomysium antibodies or anti-tissue transglutaminase antibodies.

Table 17 specifies the nomenclature codes used for our data extraction on serologic tests specifically performed for the diagnosis of coeliac disease in Belgium as well as the reimbursed amount per test.

Table 17 – Nomenclature codes for serologic tests in the diagnosis of coeliac disease

Nomenclature code		Reimbursement per test in 2024
556231	Search for anti-deamidated gliadin	3.46€
556242	antibodies, anti-endomysial antibodies, or anti-tissue transglutaminase antibodies (Maximum 2)	

Translated from :

https://www.inami.fgov.be/SiteCollectionDocuments/nomenclatureart24_20230901_01.pdf (FR)/

https://www.riziv.fgov.be/SiteCollectionDocuments/nomenclatuurart24_20230901_01.pdf (NL)

Table 18 shows that the vast majority of testing for coeliac disease in Belgium is limited to serology, with an increase of 67% in this type of testing over the period 2012-2019 and a slight decrease over the COVID pandemic years.

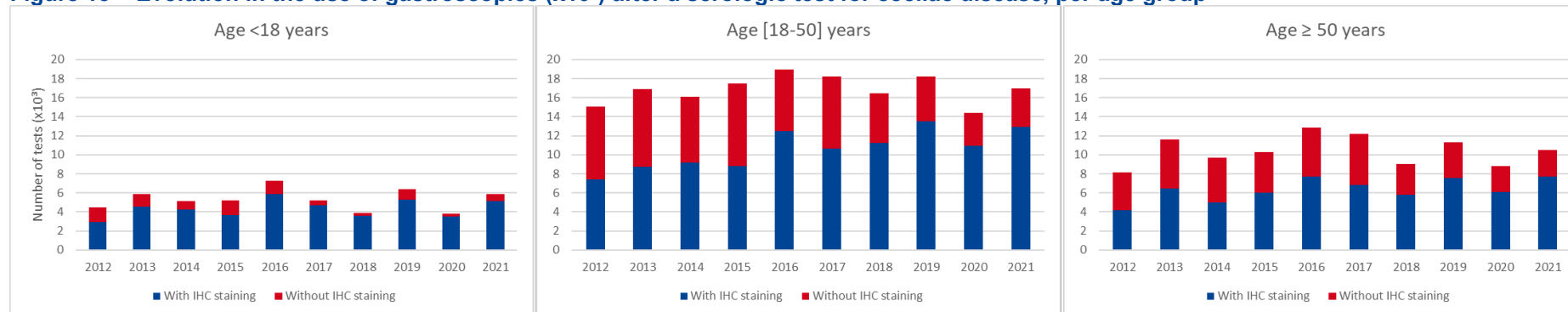
The number of gastroscopies that followed serology tests have also grown, by 30% from 2012-2019, while the proportion of IHC staining performed on the biopsy has increased at a higher rate across the years (82%). The main prescribers are gastro-enterologists (around 50% of the tests for the period 2012-2021).


Table 18 – Evolution in the number of non-invasive and invasive tests (x10³) for the diagnosis of coeliac disease (EPS 2012-2021)

	2012	2014	2016	2017	2018	2019	2020	2021	Increase from 2012-2019 (%)
Serology	166.2	217.1	253.8	258.6	266.2	277.9	238.5	245.9	67.0%
Serology + Gastroscopy (3 months after serology)	27.6 (16.6%)	30.9 (14.2%)	39.0 (15.4%)	35.7 (13.8%)	29.4 (11.0%)	35.9 (12.9%)	27.0 (11.3%)	33.3 (13.5%)	29.8%
Serology + Gastroscopy + IHC	14.5	18.5	26.1	22.2	20.6	26.4	20.6	25.8	81.7%

The evolution in the number of gastroscopies (preceded by a serologic test) is shown in (Figure 13). Differences in trends can be seen depending on the age group. Thus, there are two times less gastroscopies in the group aged <18 (~5 000 per year) compared with patients aged 18-49, where the number of gastroscopies is about 17 000 per year.

In 2019, IHC staining for coeliac disease was performed in 82.5% of the gastroscopies in the group aged <18, while in the others, IHC staining was less frequent (74.3% and 67.2% in 2019 for the groups aged 18-49 and ≥50 respectively).

Figure 13 – Evolution in the use of gastroscopies (x10³) after a serologic test for coeliac disease, per age group




Conclusion – Diagnosis of coeliac in Belgium

- In 2019, coeliac disease appears to be among the most common non-oncologic diagnostic indications for IHC in Belgium.
- Diagnosis of coeliac disease is based on a combination of clinical, serological and histopathological data. The majority of testing for coeliac disease in Belgium is limited to serology, and trends show that the use of this type of testing is increasing (+ 67% from 2012 to 2019).
- Although the number of gastroscopies and the number of gastroscopies followed by an IHC test are relatively low compared to serological testing, these have also increased over the years. While gastroscopies increased at a lower rate than serological testing, IHC use grew at a faster pace. IHC was performed in around 53% of gastroscopies that followed serological testing in 2012, but this proportion went up to 74% in 2019.
- The proportion of gastroscopies followed by IHC, appears to be higher in the younger age groups (83% in the aged <18; 74% in the aged 18-49 and 67% in those aged ≥ 50).

2.2.10 Results from the survey to Belgian laboratories – Immunohistochemistry testing

Response rate

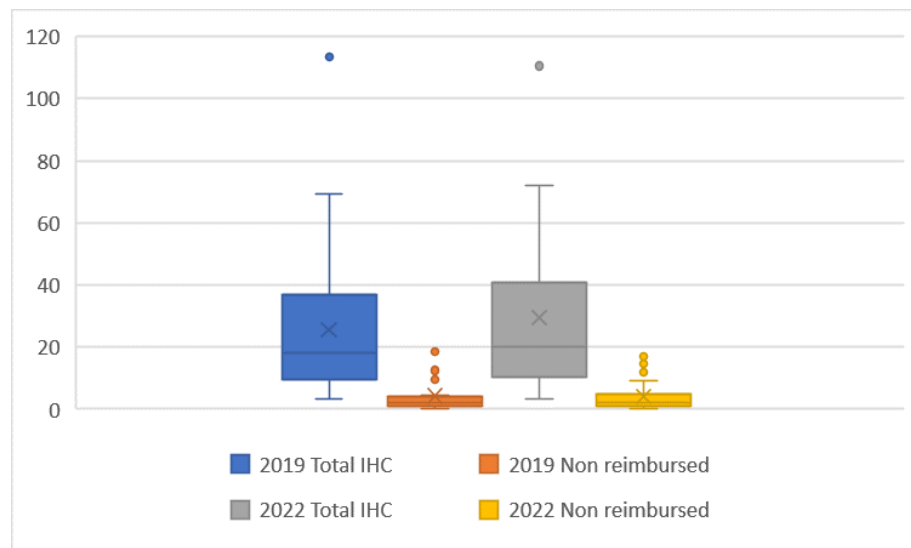
For IHC, a total of 34 responses from the 65 Belgian laboratories carrying out IHC in Belgium in 2023 were received, (25 in Dutch and 9 in French). The response rate was therefore 52%.

Number of IHC tests:

Figure 14 shows the overall number of IHC stains performed in the years 2019 and 2022, as well as the number of such tests that were not reimbursed in those same years, as reported by the respondents to our survey. Overall, the number of reported IHC stains are similar to the numbers captured by the NIHDI and can thus, be considered representative of Belgian use. Regarding non-reimbursed IHC stains, respondents to the survey reported a proportion of non-reimbursed IHC stains reaching 15% in 2019, and diminishing slightly to approximately 13% in 2022. The small reduction is likely to be a consequence of the change in nomenclature that took place in 2021, year in which separate nomenclature codes were created (i.e. 587090-587101 for lymphoid tumours and tumours of the central nervous system, and 587075-587086 for soft tissue tumours, mesothelial tumours or investigation of non-oncological pathology of kidney or liver). This was pointed out by a small number of respondents (n=3) who specifically mentioned the increase from 2021 in the number of reimbursed IHC tests for lymphoma.



Figure 14 – Distribution of the number of IHC stains performed in Belgium in 2019 and 2022 in survey participant laboratories



When asked about the main reasons why a small proportion of these tests were not reimbursed, respondents provided the following reasons:

- The numbers of diagnostic IHC stains that are reimbursed is limited to 4, which in some specific cases, is too restrictive. Thus, for certain indications, (international) guidelines recommend to perform more than 4 IHC to facilitate an appropriate diagnosis (n=32). This is also a problem when the same stain is applied to different samples from the same biopsy (n=3) or in case of technical problems (false negative or positive results), which require repeating the stain (n=2).
- IHC that are performed for quality checks are not reimbursed (n=7).
- There is no reimbursement of IHC on cytologic sampling (n=1).

Most frequent IHC stains

Participants to our survey were requested to list in order (from most frequent to least) the 5 most commonly performed IHC stains in their laboratory. Practically all respondents, listed *H. pylori* as one of the five most commonly performed stains in their laboratories (n=32 of a total of 34). From these, 23 mentioned it was the most frequent IHC stain carried out in their laboratory, with a further 7 placing it second. KI-67, on the other hand, used to assess tumour cell proliferation, was performed in all laboratories, and tended to be the second or third in terms of frequency.

Other stains proved to be less commonly mentioned with broad spectrum cytokeratin (CK-BS) being mentioned in 22 occasions, P16 in 14, and CD3 and P53, listed by 13 respondents. Finally, P63 was used by 11 respondents. Table 19 summaries the stains most commonly cited by the laboratories as well as the indications they used them in.

The remaining stains captured via our survey, were brought up by few respondents (ranging from 4 to 1). Despite the voluntary nature of our survey and the limited response rate, our findings regarding the main stains used in 2019, appear to be in line with the picture shown by the NIHD data of 2019, which indicate a good representativity of our survey results in that regard. See Table 19 for more details.



Table 19 – IHC Stains cited by the laboratories (respondent of the survey) as the 5 most frequent

IHC stain	1st most frequent	2nd most frequent	3th most frequent	4th most frequent	5th most frequent	Total	Most common reported indications
Ki67	4	15	10	3	2	34	- Proliferation index - Used for grading tumors - Cervical dysplasia, breast cancer diagnosis, melanocyte lesions, neuro-endocrine or oesophageal tumours
<i>H. pylori</i>	23	7	1		1	32	- Used for <i>H. pylori</i> detection, gastritis and stomach inflammation, to confirm the picture seen in histology, - Used to confirm eradication of <i>H. pylori</i>.
CK-BS	5	7	3	5	2	22	- Confirming the epithelial nature of a tumour in a differential diagnosis with a lymphoma, sarcoma, sentinel lymph nodes, etc. - To detect presence of metastases, micro metastases in sentinel lymph nodes - Carcinoma or prostate tumour - To see residual tumour after neo-adjuvant therapy
P16		1	3	3	7	14	- Used in cervical dysplasia, endometrium pathologies, melanocytic lesions, mesothelioma, gynaecological tumors. - To detect high risk HPV - Classification spino/ORL
CD3		2	4	4	3	13	- Diagnostic of coeliac disease - Lymphoproliferative pathology, lymphoma
P53			4	5	4	13	- Used in case of dysplasia, ovarium cancer, gastrointestinal and hepatic diseases (in Barrett mucosa, IBD (reactive vs dysplasia) and biopsies thv papilla of Vater) - Detection of P53 mutation for typing tumours (e.g. high-grade serious ovarian carcinoma, pancreatic, urology)
P63	1		3	3	4	11	- Invasive status - Make differentiation between benign, reactive, dysplasia and (pre)malignancy - Prostate or lung cancer: differential diagnosis
Melan-A			3	1		4	- Melanocyte lesions - Pigmentation lesions
ER			2		1	3	- Prognostic factor - Diagnostic exploration whether tumour of gynaecologic or breast origin
HER2					3	3	- Prognostic factors in breast cancer, but also stomach and oesophagus
Other stains*	1	2	1	11	8	23	

*The Detail of other IHC stains cited by laboratories as the 5 most frequent IHC stains can be found in an Appendix



Guidelines, protocols or literature on IHC for *H. pylori*.

Most participants to our survey (n=15), mentioned there were no guidelines or protocols describing the use of IHC staining for the diagnosis of *H. pylori*, while six mentioned such guidelines or protocols existed and eight could not tell.

Among the respondents that mentioned the existence of guidelines and protocols, three mentioned that consensus existed with regard to the use of IHC in *H. pylori* and that such consensus was based on literature but no references were provided.

A further participant, stated that a protocol for using IHC for *H. pylori* was part of their Service Level Agreement (SLA), a type of signed cooperation contract between two services. However, this SLA was at the time of the survey, under revision in order to ensure it was still up to date.

Three respondents mentioned specific sources.¹³⁻¹⁶ These relevant sources as well as any other pertinent reference included in them, were checked to see if they were picked up by our literature searches, and if not, they were added to our study selection for consideration. The results of the literature review on *H. pylori* are presented in Section 3.2.

Description of main reasons or the processes that lead to the use of *H. pylori* IHC staining in the lab:

The main reasons mentioned by participants to our survey to investigate *H. pylori* included a specific request of the clinician (n=7), together with the fear to miss any *H. pylori*, given the serious clinical consequences this bacteria may have (n=2): *"It is the only marker to demonstrate H. pylori. If this marker is not performed routinely on every stomach, mistakes will be made. Not using IHC staining in this indication is poor clinical practice!"*.

In addition to these, the fact that *H. pylori* is recognised as the main cause of gastritis and that once diagnosed, it can be easily treated, was also raised (n=5).

When asked to describe the processes that lead to the use of IHC staining for *H. pylori* in their laboratories, most respondents focused their answers

on the need to explore the presence of *H. pylori*, but not on the specific choice of test to do so. Those who offered an insight into the basis for their test choice, reported IHC to be a particularly helpful tool in cases where difficulties in identifying *H. pylori* with H&E are experienced (e.g. cases in which an absence of *H. pylori* was coupled with an inflammation of the mucosa) (n=3). Others indicated that gastroenterologists request a *H. pylori* test on the large majority of gastric biopsies and IHC is recognised as the international gold standard method for effectively detecting Helicobacter gastritis (n=2).

Four participants specified that IHC staining for *H. pylori* was performed when specifically requested by the physician, or when the request made by the physician required IHC staining to be appropriately addressed.

Five respondents reported using IHC staining to check the eradication of the *H. pylori* infection, one of whom specified this was done only for children.

Finally, two participants mentioned IHC staining to be routinely done in the laboratory on any biopsies from gastroscopies, since this was considered the most appropriate technique to diagnose *H. pylori* (by both gastroenterologists and pathologists).

In general, identifying *H. pylori* with other techniques was thought to be difficult in many cases.

Guidelines, protocols or literature on CD3

According to the survey respondents, CD3 is mainly used for coeliac disease (n=9), lymphoma (n=7) or lymphoproliferative pathologies (n=6).

Regarding the existence of guidelines for its use in coeliac disease, five out of the 9 participants who identified it as one of their most common IHC staining indication used in their laboratory, reported a lack of guidelines, while one highlighted the existence of guidelines and gave a reference¹⁷ that will be considered in the literature review for coeliac disease (see section 3.3). A further one could not tell and the remaining did not answer the question.



With regards to guidelines or protocols on the use of CD3 in lymphoma, only one respondent stated that guidelines were available, but did not provide any specific references.

Similarly, for CD3 use in lymphoproliferative pathologies, one participant mentioned guidelines, but no supporting documents were provided.

When asked to describe the main reasons or the processes that lead to the use of CD3 staining for these common indications, most laboratories gave very general reasons, without describing in detail the decision-making process. Answers included:

- Based on the clinical question, it is decided whether the CD3 staining is relevant to arrive at a diagnosis (n=2)
- CD3 is performed if specifically requested by the doctor, or if the tissue is suspicious for coeliac disease.
- Gastroenterologists request a CD3 stain on every duodenal biopsy to determine the number of intraepithelial lymphocytes and thus, demonstrate or rule out coeliac disease.
- The decision to perform CD3 is based in internal agreements.
- CD3 is used when there is a suspicion of increased intraepithelial lymphocytosis on small intestine or colon biopsies. This is standard practice in duodenum biopsies of paediatric patients.

Guidelines, protocols or literature on IHC staining for tumour cell proliferation (Ki67)

Despite the fact that Ki67 can be used in different types of tumours, and given that the survey requested information on the specific indications for which IHC staining is most frequently performed, most respondents identified breast cancer (n=14) and cervix dysplasia (n=14) as the main fields where they use such IHC stain, while other less common indications included neuroendocrine tumours (n=6), Barrett oesophagus (n=5), and

lymphoma (n=4). Some participants gave tumour cell proliferation in general as their most common indication (n=6) or mentioned Ki67 to be very helpful at grading different tumours (n=3).

Regarding the existence of guidelines or protocols for the use of Ki67, half of those respondents that reported breast cancer as one of their five most common IHC staining indications, mentioned the existence of guidelines for its use (n=7), while three reported a lack of guidelines (one of which specifically mentioned a lack of “internal” guidelines or protocols). Two did not answer the question and the remaining could not tell.

Similarly, for Ki67 in cervix dysplasia, seven participants who identified it as one of their five most common IHC staining indication, cited the existence of guidelines or protocols for its use, while a further three could not tell and one did not answer.

The existence of guidelines or protocols for the other less frequent indications was also stated by some of the respondents:

- For neuroendocrine tumours: 4, while one did not know and the last respondent did not answer.
- For Barrett oesophagus: 2, while a further two stated there were no guidelines, and one could not tell.
- For lymphoma: 2, while one mentioned there were no guidelines, one could not tell and the remaining did not answer.

Some participants mentioned they use it more to guide therapeutical decisions, particularly in breast cancer, but also in other tumours.

In general, respondents who reported the existence of guidelines did not always provide the relevant references, and stated instead that guidelines and literature from professional groups, as well as reference books, existed.

Table 20 lists all references provided for Ki67 staining in the different indications:

**Table 20 – Guidelines or references mentioned by respondents for the use of Ki67 in different indications**

Author/organisation	Source	Available at
College of American Pathologists	Guidelines by CAP	https://www.cap.org/protocols-and-guidelines
WHO	Classification of WHO	https://tumourclassification.iarc.who.int/welcome/
ESMO	Cancer guidelines ESMO	https://www.esmo.org/guidelines
ESMO - Pavel M, Öberg K, Falconi M et al.	Gastroenteropancreatic neuroendocrine neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020 Jul;31(7):844-860.	https://pubmed.ncbi.nlm.nih.gov/32272208/
ASCO	Cancer guidelines ASCO	https://ascopubs.org/jco/special/guidelines
NCCN	Clinical guidelines NCCN	https://www.nccn.org/
Pathologyoutlines.com	General review with references provided	https://www.pathologyoutlines.com/
Ki67 in Breast Cancer Working Group	Nielsen TO, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2021 Jul 1;113(7):808-819.	https://pubmed.ncbi.nlm.nih.gov/33369635/
International collaboration for cancer reporting	International collaboration on pathology reporting	https://www.iccr-cancer.org/?s=Ki+67
Clement P B, Young R, H.	Atlas of Gynaecologic Surgical Pathology. 2008; 2nd Ed	NA



Description of main reasons and processes why Ki67 staining is performed in different indications in the laboratory:

According to the respondents, the main reasons or processes that lead to the use of Ki67 IHC staining for different indications remained rather general and included the following:

- The proportion of positive cells plays a role in any therapy decisions (e.g. in neuroendocrine tumours and breast carcinomas), but are also diagnostically important in, for example, lymphomas and at determining degrees of tumour differentiation.
- Ki67 is used as an aid to help pathologists in making a diagnosis by for example answering questions such as: is there dysplasia or not? Or is a tumour benign or not? in different tumours (n=6).
- The Ki67 IHC stain shows the proliferation rate of the cells in tumours (proliferation marker) and is widely used internationally to classify tumours (benign/malignant? type?). The degree of proliferation is also important in benign tissues, for example to grade dysplasia (mild/moderate/severe). Pathologists must decide on a case-by-case basis whether staining is useful.
- In breast cancer, Ki-67 is a crucial clinical parameter.
- Specifically for breast cancer, Ki67 is routinely asked and results are registered as part of the MOC, since Ki67 labelling index is taken into account when choosing treatment options (n=3).

Guidelines, protocols or literature on CK-BS.

CK-BS is a stain used in different indications. The most common use in Belgium is, according to our questionnaire, in breast cancer and gynaecological tumours. However, most respondents to the survey who mentioned CK-BS as one of their most common IHC stains, referred to a general use in epithelial tumours or carcinoma as the key indication for this stain (n=13). Very few stated that they employ it in very specific indications such as prostate (n=2), lymphoma (n=1) or bladder (n=1) tumours, while four reported using it for diagnosing different types of cancers (n=4) and five for studying metastases (n=5).

Regarding the existence of guidelines for the use of CK-BS, ten respondents knew of available guidelines, most in breast cancer, but also in gynaecological and oral tumours. Thirteen thought there were no guidelines available, while five could not answer the question and a further five did not know. Table 21 lists the sources specifically provided by the participants.

**Table 21 – Guidelines or references mentioned by respondents for the use of CK-BS in different indications**

Author/organisation	Reference	Available at
Cserni G.	Reporting of Surgically Removed Lymph Nodes for Breast Tumors: Recommendations From the International Collaboration on Cancer Reporting. Arch Pathol Lab Med. 2022 Nov 1;146(11):1308-1318	https://pubmed.ncbi.nlm.nih.gov/36270029/
Cserni G.	Sentinel lymph node assessment in breast cancer-an update on current recommendations. Virchows Arch. 2022 Jan;480(1):95-107	https://pubmed.ncbi.nlm.nih.gov/34164706/
Biganzoli L.	The requirements of a specialist breast centre. Breast. 2020 Jun;51:65-84	https://pubmed.ncbi.nlm.nih.gov/32217457/
Peintinger F.	National Breast Screening Programs across Europe. Breast Care (Basel). 2019 Dec;14(6):354-358.	https://pubmed.ncbi.nlm.nih.gov/31933580/
International Collaboration on Cancer Reporting	Published Datasets - Breast	https://www.iccr-cancer.org/datasets/published-datasets/breast/
Schilling C.	Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. Eur J Cancer. 2015 Dec;51(18):2777-84.	https://pubmed.ncbi.nlm.nih.gov/26597442/
Cibula D.	ESGO/ESTRO/ESP Guidelines for the management of patients with cervical cancer - Update 2023. Int J Gynecol Cancer. 2023 May 1;33(5):649-666.	https://pubmed.ncbi.nlm.nih.gov/37127326/
Colpaert C.	Belgian Working Group for Gynecological Pathology. Ultrastaging of sentinel lymph nodes in gynecological cancer: Repeating the story of breast cancer? Letter to the editor, Reply to Cibula D, McCluggage WG. Sentinel lymph node (SLN) concept in cervical cancer: Current limitations and unanswered questions. Gynecol Oncol 2019;152:202-7. Gynecol Oncol Rep. 2019 Feb 15;29:130-131.	https://pubmed.ncbi.nlm.nih.gov/31517013/
Dabbs J.	Diagnostic immunohistochemistry, Theranostic and Genomic Applications; 6th Edition - November 23, 2021; Editor: David J Dabbs; ISBN: 9780323721721	NA
Pathology Outcomes.com	Stains & CD markers Cytokeratin AE1 / AE3	https://www.pathologyoutlines.com/topic/stainsae1ae3.html
Kato S.	Cancer of Unknown Primary in the Molecular Era. Trends Cancer. 2021 May;7(5):465-477.	https://pubmed.ncbi.nlm.nih.gov/33516660/
Lyman G H.	American Society of Clinical Oncology Guideline Recommendations for Sentinel Lymph Node Biopsy in Early-Stage Breast Cancer. J Clin Oncol 2005; 23(30):7703-7720	https://pubmed.ncbi.nlm.nih.gov/16157938/



Description of main reasons why CK-BS staining is performed in different indications:

Respondents gave general reasons why they use this stain in their laboratory. These included mainly the recognised usefulness of the stain, as well as the crucial role of the pathologist in deciding when such a test should be performed, on a case by case basis:

- The use of coloring for this purpose is 'obvious' but it is not imposed by guidelines.
- Every pathology textbook mentions the usefulness of this stain in assessing the differential diagnosis of carcinoma - lymphoma – melanoma.
- Every textbook of general pathology and uropathology mentions the usefulness of this stain, there is no 'guideline' that imposes this, but the use of the stain is left to the expert judgment of the pathologist.
- Used to assess if there is an epithelial tumor or not.
- Used to check if there is a residual carcinoma or not.
- Used to check whether a biopsy shows evidence of an epithelial tumor, before further differentiating the tumor.
- Used to control for lymphadenectomy.
- Used for determining the depth of invasion.
- Used for performing architectural assessments (e.g. chest cylinder).
- Used in general in the context of cancer diagnosis.
- Cytokeratin marks the architecture of lesions and epithelial cells not present in the stroma.
- Based on the clinical question, it is decided whether the CK-BS staining is relevant for making a diagnosis.
- There are many different indications for this IHC stain. Each pathologist must decide on a case-by-case basis whether the staining is useful or necessary to make a correct diagnosis. For example, in stomach biopsies: in case of dense inflammation to exclude signet ring cell carcinoma; while with little inflammation, this is not necessary (i.e. it is clear enough on H&E staining).
- The assessing pathologist decides to perform this stain to determine whether or not it is a carcinoma (often in combination with other IHC stains).

Guidelines, protocols or literature on P16.

The main indication according to the respondents who mentioned P16 as being one of the five most frequent stains performed in their laboratories was cervix dysplasia (n=13). A further less common specific indication captured was melanoma (n=3). Finally, seven participants reported a more general use in endometrial tumours and other gynaecologic lesions (n=5) and in oropharyngeal tumours (n=2).

Regarding the existence of guidelines or protocols for the use of P16 specifically for cervix dysplasia, more than half of the respondents mentioned guidelines existed, two said there were none and three could not tell. From the five who reported more general use on endometrial and other gynaecologic tumours, four respondents reported a lack of guidelines, while two mentioned that guidelines were available. For melanoma, one respondent reported that guidelines were available, while two mentioned there were none. Finally, for oropharyngeal tumours, one respondent highlighted the availability of guidelines while the other could not tell.

Table 22 lists the references given for guidelines or protocols for P16 IHC staining in the previously mentioned indications.

**Table 22 – Guidelines or references mentioned by respondents for the use of P16 in different indications**

Author/organisation	Reference	Available at
Clement & Young	Atlas of Gynaecologic Surgical Pathology., 2nd Ed. (cervix dysplasia)	NA
ESMO	Cancer guidelines ESMO	https://www.esmo.org/guidelines
ASCO	Cancer guidelines ASCO	https://ascopubs.org/jco/special/guidelines
NCCN	Clinical guidelines NCCN	https://www.nccn.org/
WHO guidelines (cervix dysplasia)	WHO Classification of Tumours of Female Reproductive Organs. 4th Edition, Volume 6 Edited by Kurman RJ, Carcangiu ML, Herrington CS, Young RH	https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Female-Reproductive-Organs-2014
College of American Pathologists and the American Society for Colposcopy and Cervical Pathology	Darragh TM et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012 Jul;16(3):205-42	https://pubmed.ncbi.nlm.nih.gov/22820980/

When asked to describe the reasons or the processes that lead to the use of P16 IHC staining for the previously mentioned indications, most laboratories gave very general reasons, without describing in detail the decision process. Answers included:

- When there is suspicion of dysplasia, or if requested by the clinician.
- No guideline imposes its use, but every gynaecological pathology textbook, as well as the WHO classification of tumours books (known as the blue books) mention the usefulness of this specific stain. Thus, its use in specific cases is left to the expert judgment of the pathologist.
- There are several different indications for this IHC stain. Each pathologist must decide on a case-by-case basis whether the staining is useful or necessary to make a correct diagnosis (eg. in cervical biopsies: to grade dysplasia, when it is not clear enough on the H&E stain).

Guidelines, protocols or literature on P53.

The most common indications mentioned by the respondents who reported to use P53 staining included endometrial and other gynaecologic tumours not specified by the respondents (n=5), ovarian cancer (n=4), dysplasia (n=4), Barrett's oesophagus and liver diseases (n=4).

Regarding the existence of guidelines for the use of P53 in ovarian cancer, three of the four participants mentioning this indication specifically reported the existence of guidelines, while only one mentioned there were none. A similar picture was drawn for responses obtained for endometrial tumours and other gynaecological malignancies. Four respondents recognised that guidelines were available and only one said there were none. Most respondents mentioning Barrett's oesophagus and liver tumours also highlighted the existence of guidelines (n=3). Dysplasia was the only use, from those mentioned where most participants, with the exception of one, reported a lack of guidelines (n=3).

Table 23 offers information on the specific references given for guidelines or protocols for P53 in the previously mentioned indications:

**Table 23 – Guidelines or references mentioned by respondents for the use of P53 in different indications**

Author/organisation	Reference	Available at
WHO	Classification of WHO	https://tumourclassification.iarc.who.int/welcome/
Dabbs J.	Diagnostic immunohistochemistry, Theranostic and Genomic Applications; 6th Edition - November 23, 2021; Editor: David J Dabbs; ISBN: 9780323721721	NA
Pathologyoutlines.com		https://www.pathologyoutlines.com/topic/stainsp53.html
Weunsten B et al.	Diagnosis and management of Barrett esophagus: European Society of Gastrointestinal Endoscopy (ESGE) Guideline	https://www.bsg.org.uk/wp-content/uploads/2023/12/ESGE-Barretts-Guideline.pdf
Fitzgerald RC et al.	British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut. 2014 Jan;63(1):7-42.	https://www.bsg.org.uk/clinical-resource/bsg-guidelines-on-the-diagnosis-and-management-of-barretts-oesophagus/
ESMO	Cancer guidelines ESMO	https://www.esmo.org/guidelines
ASCO	Cancer guidelines ASCO	https://ascopubs.org/jco/special/guidelines
NCCN	Clinical guidelines NCCN	https://www.nccn.org/

When asked to describe the reasons or the processes that lead to the use of P53 IHC staining for the above mentioned indications, most laboratories gave general reasons, without describing in detail the decision process. Answers included:

- Strong expression or complete loss is an argument in favour of dysplasia.
- Distinguish/differentiate between benign, reactive, dysplasia and (pre)malignancy, for example in oesophagus, skin, bladder, ...
- Use decided based on internal agreement (e.g. Barrett's oesophagus).
- The assessing pathologist decides to perform this stain to determine dysplasia.
- Used for the assessment of aberrant staining – dysplasia grading.

Other points captured via the survey:

Respondents raised some general points they considered important. These included the following:

Sources frequently consulted by pathologists:

- In addition to the WHO blue books – important references are summarised in two frequently consulted websites: <https://www.pathologyoutlines.com/> and Kurt's notes (<https://schaberg.faculty.ucdavis.edu/>). The first of these was commonly mentioned in our survey as a helpful source for decision making in this field.
- Reference books exist on different pathologies. In addition to these, annual conferences and accredited training are other important sources for Belgian pathologists.



- A further important book often consulted in general for IHC is: Diagnostic Immunohistochemistry: theragnostic and genomic applications (fifth edition 2019), author David J. DABBS, publisher Elsevier Philadelphia PA USA. This is already captured in the tables previously presented, specifically for P53 and for CK-BS.

Need for guidelines:

- A mention was made that IHC stains have become in the last years a permanent and indispensable part of good clinical practice, necessary for an effective and complete diagnosis in different medical areas, and that since clear guidelines and protocols are lacking in some of these areas in Belgium, there is room and need for these, to facilitate decision-making by pathologists and reduce variation in use across Belgian laboratories.

Reimbursement limitations to four IHC stains:

- Some laboratories facing many oncological cases, require under some circumstances more than four stains to make a clear diagnosis. Moreover, clinicians are increasingly requesting additional IHC examinations or wanting IHC confirmations (e.g. vascular emboli, MSI, ki67, p16, etc.), which can no longer be reimbursed.
- Certain IHC stains should be moved from diagnostic to prognostic (e.g. like mlh1,mlh2....)
- Due to the ever-increasing number of very specific IHC stains, the reimbursement of maximum four IHC stains per patient is outdated.
- A maximum of four reimbursed IHC stains is insufficient in many cases of complex diagnostics (the WHO Blue books increasingly set requirements for diagnostics). This means pathologists themselves have to cover for the cost of carrying out necessary non-reimbursed tests. Non-reimbursed IHC stains accounted for around 15% of all IHC stains in 2019 and for 13% in 2022.

Critics to the survey:

- A respondent though the questionnaire contained too many ambiguities, and explained that people are often asked about guidelines, but IHC stains are not simply requested for 'every case of ...' nor according to an exhaustive list, but in specific cases to resolve a differential diagnosis. This participant believed a judicious request of these stains by a skilled pathologist offers many more guarantees against 'overconsumption' than guidelines.
- A further respondent found the survey to offer an approach too narrow for evaluating indications for IHC and the use of this technique. This point was made despite the fact that a short description of the overall research project was offered in the introductory email to the laboratories, explaining the survey was only a part of the overall research here presented.



Conclusion – Survey results - The use of diagnostic IHC stains in Belgium

- The mean number of reported IHC stains performed in 2019 was according to the respondents 25 452, compared to a mean of 17 659 for that same year using the extrapolated data from the EPS. This appears to indicate that there may be a possible participation bias towards laboratories performing a higher volume of IHC staining.
- The mean number of IHC stains done per laboratory appeared to have increased by approximately 15% from 2019 to 2022, indicating that the grow in use of these stains continued after the COVID pandemic years.
- According to the survey respondents, non-reimbursed IHC stains, reached 15% in 2019 and 13% in 2022. The main reasons mentioned by respondents why there were some stains that were not reimbursed was the current reimbursement limitation to four stains, which in some cases were considered insufficient.
- The main IHC stains used in Belgium and the associated reported indications included *H.pylori* (32/34 respondents), Ki67 for tumour cell proliferation in different tumour types (34/34), CK-BS for cancers of epithelial origin (22/34), P16 for cervix dysplasia, melanocytic lesion, endometrium pathology,... (14/34), CD3 for celiac disease, lymphoproliferative pathology or lymphoma (13/34) and P53 for ovarian cancer, Barrett oesophagus, etc (13/34).

- The main reasons for the use of these IHC stains in the different indications was linked to their accuracy and the difficulty to offer an appropriate diagnosis purely based on H&E. The specific request of the clinician was also mentioned by the respondents.
- Not all respondents could tell if guidelines for the use of these stains were available. Responses varied greatly depending on the indications, but overall, only a minority of respondents gave some specific references on this regard.

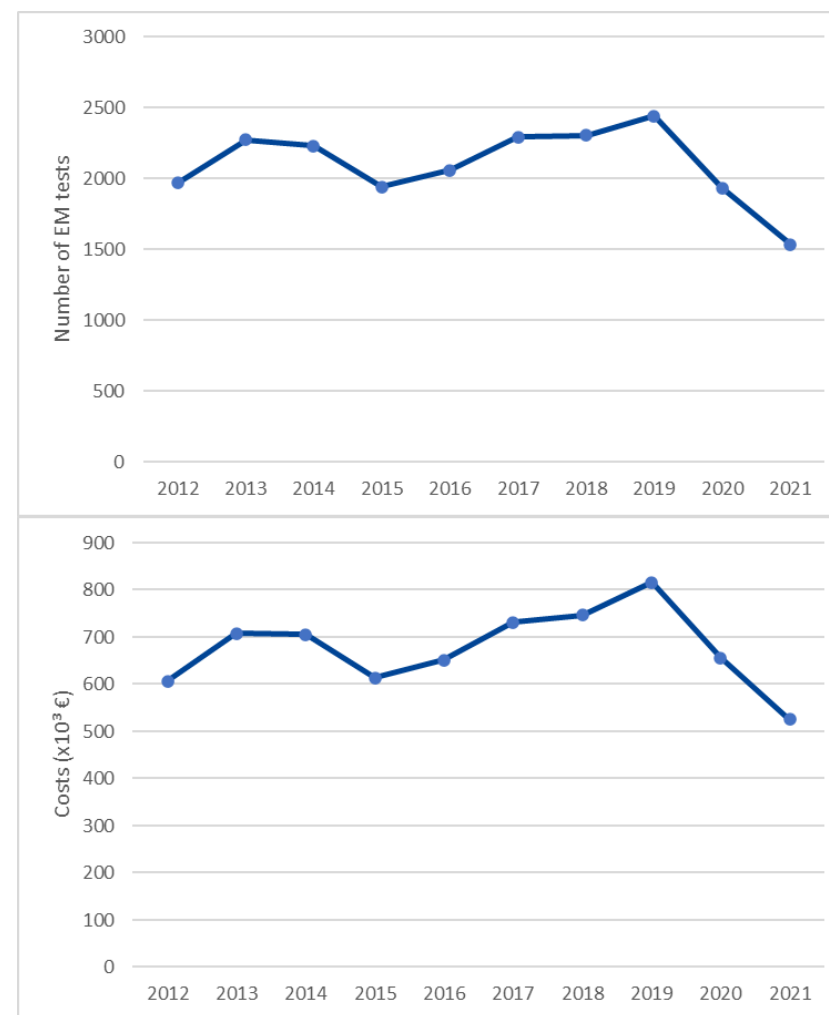
2.3 Results: Electron Microscopy testing

2.3.1 General evolution in use of electron microscopy testing

Table 24 and Figure 15 show the number of EM tests performed between the years 2012 and 2021. These vary from, a low of 1534 in 2021 and 1934 in 2020 (the years of the COVID pandemic), to a high of 2293 in 2017. From 2012, to 2021, the number of tests decrease by 22%, while excluding the years of the pandemic, an increase of 24% is seen from 2012 to 2019. The associated costs follow the same trend. Although it is unclear if the decreasing trend seen during the pandemic period is likely to continue, most of the previous years show the use of these tests to fluctuate between 2000 and 2500 annual tests.

**Table 24 – Evolution in use of EM testing (Source: Doc N)**

Year	Number of tests	Costs (x10 ³ €)
2012	1968	606,2
2013	2276	707,1
2014	2232	705,0
2015	1942	613,7
2016	2059	651,0
2017	2293	731,5
2018	2306	746,2
2019	2442	815,9
2020	1934	655,8
2021	1534	524,7

Figure 15 – Evolution in use of EM testing (Source: Doc N)



2.3.2 NIHDI Data Analysis – Electron Microscopy

Reports for all EM diagnostic tests performed in the year 2019 were summarised by the NIHDI in an anonymised excel sheet transferred to the KCE team for analysis. A total of 2432 EM tests for the diagnosis of 2355 patients were performed in 2019 for a total amount of 813 782€. Eleven laboratories performed this type of diagnosis in Belgium. However, for two of these, no information was reported on indications, and thus, these were excluded from our analysis. Table 25 summarises the number of tests, per indication and by laboratory.

Table 25 – Tissue analysed for diagnosis by EM in Belgium in 2019, by laboratory

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Total
Kidney	26 (2.9%)	0	295 (33.1%)	0	42 (4.7%)	155 (17.4%)	112 (12.6%)	184 (20.7%)	77 (8.6%)	891
Skin	0	0	2 (0.3%)	22 (2.9%)	0	2 (0.3%)	724 (96.2%)	0	3 (0.4%)	753
ENT	0	0	1 (0.4%)	4 (1.6%)	0	241 (97.6%)	1 (0.4%)	0	0	247
Muscle	43 (21.7%)	0	4 (2.0%)	23 (11.6%)	0	53 (26.8%)	52 (26.3%)	0	23 (11.6%)	198
Lung - BA Lavage	0	125 (97.7%)	0	0	0	0	3 (2.3%)	0	0	128
Platelet assessment	0	0	64 (100%)	0	0	0	0	0	0	64
Sputum smear - Amiodarone Pulmonary Toxicity	0	0	0	14 (100%)	0	0	0	0	0	14
Meningioma/ hypophysis	0	0	0	35 (68.6%)	0	0	16 (31.4%)	0	0	51
Nerve	2 (9.1%)	0	0	3 (13.6%)	0	15 (68.2%)	2 (9.1%)	0	0	22
Total	71 (3.0%)	125 (5.3%)	366 (15.5%)	101 (4.3%)	42 (1.8%)	466 (19.7%)	910 (38.4%)	184 (7.8%)	103 (4.4%)	2368

BA=broncho-alveolar, 64 EM (including 2 labs) were removed because of ≤10 EM performed/indications) or no information on the indications.



Kidney biopsies were by far the most commonly analysed specimens via EM. Seven out of the nine laboratories carried out this type of investigation. The second and third most commonly analysed tissues were skin, and ENT (mostly cilia) although their analysis was almost exclusively carried out in a single laboratory. Muscle biopsies were the fourth most commonly analysed tissue type, and were carried out by six laboratories.

Kidney indications: in 81% of cases, IHC was performed in addition to EM. In these cases, more than four stains (the maximum reimbursed by the NIHDI) were used, and the types of antigens were specific to glomerular nephropathies (e.g. systemic lupus erythematosus characterised by deposits of some antigens and visible by EM).

Skin indications: in the laboratory performing practically all EM testing for this type of indication (Lab 7 in Table 25), this is done in 88% of cases in combination with IHC, using the antibody Xilla (637/724 EM). This test is specific for research in Ehlers-Danlos syndrome. It differs from single IHC testing on skin biopsies in the absence of any EM testing often performed for melanoma (E.g.: including the antigen Melan-A).

Primary ciliary dyskinesia: is mostly done in one laboratory (lab 6 in Table 25). The combination of EM and IHC testing is seen in very few cases (<1%).

Muscle indications aimed at diagnosing myopathies: in 82% of case, IHC is performed in parallel. As for the kidney, the number of antigens tested are often above 4.

Broncho-alveolar lavage: is performed mainly in one laboratory (lab 2 in Table 25), is carried out for the diagnosis of diffuse interstitial lung diseases, infectious or not (e.g. sarcoidosis, pulmonary fibrosis, presence of bacteria, ...). IHC testing is rarely performed as part of these diagnoses.

EM for platelet assessment is performed only in one laboratory (lab 3 in Table 25). No IHC tests are performed in parallel in this specific indication.

Sputum smear (for Amiodarone Pulmonary Toxicity): is only performed in one laboratory (lab 4 in Table 25). This EM testing is not combined with IHC in these cases.

Nerve indications: EM for these indications is performed in four laboratories, although three of these used it in three or less cases in 2019, with the 4th laboratory performing EM in 15 cases in that same year. In 91% of cases, IHC is also performed.

The limitation of the EPS being a sample of all reimbursement data, coupled with the fact that use of EM is relatively limited, impeded further analysis on other tests often performed in addition to EM.

2.3.3 Results from the survey – Electron microscopy

Overall, seven out of eleven Belgian laboratories carrying out EM responded to our survey (3 in Dutch and 4 in French). The response rate was therefore 64%, although the level of detail provided varied greatly between respondents.

Table 26 summarises the most frequent areas of use for EM according to the seven responding laboratories, which appear to match the NIHDI data, indicating a good representation of clinical practice.

Table 26 – Most frequent indications for EM, according to respondents

	1st most frequent	2nd most frequent	3th most frequent	Total
Kidney	4	3		7
Neuromuscular	2	2	2	6
Ciliary dyskinesia		1	2	3
Liver			1	1
Skin	1			1

**EM use in renal pathologies:**

From the respondents mentioning kidney diseases as being one of their most common areas of EM use, two specified performing EM in glomerulopathies, two reported using it in kidney transplantation, while the remaining mentioned general use in renal pathologies, without offering further detail.

From the seven respondents, three mentioned the existence of guidelines in this field, although only one guideline was actually provided.¹⁸ This covered the area of pathologic classification, diagnosis, and reporting of glomerulonephritis, and it will be analysed in detail in the chapter on the review of the literature for the most frequent EM indications (see section 3.4). Two other respondents did not know if guidelines were available, while the remaining two did not provide an answer to this question.

All of the laboratories using EM in this area confirmed no alternative test existed in this field, since simple histology would not be enough to offer a diagnosis thus, requiring to be supplemented by EM.

EM use in neuromuscular diseases:

From the respondents mentioning neuromuscular diseases as being one of their most common areas of EM use, three explained they use it in myopathies, one of which specifically mentioned mitochondrial myopathy as an area of interest. A further respondent referred to neurogenic or myogenic muscle disorders, while the remaining two simply stated the use EM for the diagnosis of neuromuscular diseases.

From the six respondents, only one mentioned that guidelines existed in this field, referring to international recommendations on how to manage neuromuscular biopsies. However, no specific references were provided on this regard. A further respondent stated that no guidelines existed in this field given the rarity of these diseases, but that a relevant source was the European reference network for rare and low prevalence diseases – Network Neuromuscular Diseases.ⁱ The same respondent also mentioned a study by Sommer et al., published in 2010 on processing and evaluating

nerve biopsies.¹⁹ The relevance of these sources will be explored in the chapter on the review of the literature for the most frequent EM indications. Two other respondents did not know if guidelines were available, while the remaining two, did not provide an answer to this question.

From the four laboratories that answered the question only one mentioned that an alternative to EM (specifically for mitochondrial myopathies) could be genetic testing. The other three stated that no alternative tests existed in this field, and that the use of EM was thought to be necessary for an accurate diagnosis.

EM use in ciliary dyskinesia:

From the three respondents mentioning ciliary dyskinesia as one of their most common areas of EM use, two mentioned the availability of guidelines in this field. The third one did not answer that question. Two references were provided.^{20, 21} These will be considered in the chapter on the literature review for common EM indications.

All of the laboratories using EM in this field confirmed no alternative tests existed in this area.

Other EM indications:

Two respondents reported use of EM in two different areas. The first laboratory performed EM in skin biopsies for the diagnosis of collagenopathies and did not know if guidelines existed in this field, but stated that no alternatives existed. The second respondent mentioned liver tumours and liver disease in general as an area of interest for EM. Once more, the respondent was not aware of the availability of guidelines in this area, but confirmed that no other alternatives existed.

ⁱ <https://ern-euro-nmd.eu/recommended-guidelines>



Conclusion – The use of diagnostic EM in Belgium

- A total of 2432 EM tests for the diagnosis of 2355 patients were performed in 2019 for an amount of 813 782€.
- In the period 2012-2019, the number of EM tests varied from 1900 to 2500, without a very clear trend (slight increase before the Covid years).
- The most common indications for EM diagnosis were kidney and neuromuscular diseases (in 7 and 6 Belgian laboratories respectively).
- Other indications appear to be laboratory-specific.
- Guidelines were known and reported by few of the respondents to the survey on EM use in Belgium. These concerned only kidney disease and primary ciliary dyskinesia.
- None of the respondents reported any alternative tests for the specific indications studied with the exception of mitochondrial myopathies, where genetic testing was reported to offer a possible alternative.

3 A REVIEW OF THE LITERATURE FOR IHC AND EM - FOCUS ON THE MOST COMMON INDICATIONS

This chapter presents first, the common methods used for carrying out the literature reviews pursued for both IHC and EM in the most frequent indications identified via our data analyses presented in Chapter 2 of this report. Following that, different sections in this chapter offer an overview of the results starting with IHC and then, moving onto EM.

3.1 Methods

A search of the literature limited to the most frequent indications for both IHC and EM was performed. For IHC staining, the main focus was on two non-oncologic indications: First, practice guidelines and Systematic Reviews (SRs) on *H. pylori* were searched, since our data analyses of the NIHDI data (see section 2.1.1) showed this was the most frequent indication for diagnostic IHC, and the survey to Belgian laboratories confirmed such finding. In addition to *H. pylori*, guidelines and SRs for **coeliac disease** were also explored, since this appears to also be a relatively common non-oncologic indication for IHC staining, concentrating an important proportion of use, (according to our survey to Belgian laboratories) and likely to continue growing in the future, given the high prevalence levels and the improvements in diagnosis in this field over the last years.

We excluded clear oncological IHC indications, even if mentioned in the online survey as common indications, from the literature review due to the complexity of the underlying pathologies, which would have prevented us from completing the task within the given timeframe. Nevertheless, the sources mentioned by the respondents regarding those indications are listed for completeness, in the section reporting the results of our survey (section 2.2.10) .



With regards to EM diagnoses, the focus was put on the most common indications reported in the 2019 NIHDI data (kidney, muscle, skin, and cilia), which were also the main indications captured in the survey responses.

In order to ensure an effective use of the limited resources, the search was initially limited to systematic reviews (SR), HTAs and clinical guidelines. Only if none of these types of studies were identified, comprehensive reviews were considered.

Any documents mentioned and identified by means of our survey to the Belgian laboratories were also evaluated for inclusion.

Any articles published from 2013 in English, French, Dutch, German, Italian, Portuguese, or Spanish were considered in our analysis but no a priori-language limits were imposed. The PICO's for the reviews, as well as a link to our search strategies are provided in an appendix.

3.1.1 Databases

OVID Medline, EMBASE and Cochrane databases were consulted, while the websites of other HTA agencies listed in the INHATA website^j were checked for any recent research in this field. Similarly, a search in the grey literature using general terms was performed, and Belgian laboratories were asked about important references they would recommend the research team to consider for the different indications previously specified for both IHC and EM.

For literature searches on the use of EM in the indications that consisted of groups of diseases (rather than one specific disease), such as renal and muscular pathologies, the search string included EM as one of the search terms. This approach was chosen due to limitations in time and resources, preventing individual searches for guidelines on diagnostic methods for the multitude of diseases falling under kidney and muscular pathologies.

The searches were carried out by an information specialist at different dates from September to December 2023 and are provided in the appendix of this report.

3.1.2 Study Selection

The search results from the consulted databases were exported into a Rayyan folder, and duplicates were removed. First, clinical guidelines and systematic reviews (SRs), with or without meta analyses (MA) were reviewed for inclusion. If relevant evidence was found purely based on these study types, a narrative description is presented. For EM, given the scarcity of evidence, general reviews (non-systematic) were also considered.

The first step of the study selection (on the basis of title and abstract) was performed by one researcher, but this was preceded by a pilot on the 50 most recent references identified in the RAYYAN folder, during which the selection was carried out in parallel by two researches. After completion of this pilot, results were shared and any conflict resolved via discussions. If necessary, a third researcher was consulted. Once this step was accomplished, one researcher completed the screening of the remaining manuscripts.

Full texts of any relevant or unclear studies identified via the search were inspected individually by one researcher. Reasons for exclusion of full-texts were documented and categorized according to predefined in-and exclusion criteria (see PICO's for the different searches in appendix). In case of doubt, the reviewer consulted the internal research team to determine eligibility or reason for exclusion.

The study selection processes are presented in flowcharts.

^j <https://database.inahta.org/>



3.1.3 Data extraction and synthesis

Relevant data from the included studies were extracted and tabulated in a data extraction form. This was done by one reviewer. A template for data extraction was developed, discussed (within the research team) and refined.

Tables including characteristics and results of the included studies are presented. The quality assessment of the studies is provided in an appendix.

Endnote is used as reference software when synthesizing the review results in our report.

3.1.4 Quality appraisal

To appraise the quality of guidelines retrieved the Appraisal of Guidelines, Research and Evaluation II- Global Rating Scale (AGREE II-GRS) instrument²² was used. The AGREE II GRS instrument evaluates guidelines based on their process of development, presentation style, completeness of reporting and clinical validity. It requires to give an overall quality rate based on the appraisal of the four dimensions. It is a shorter version of the AGREE II instrument²³ that may be used when time is limited. Ratings of the quality of the AGREE domains are good predictors of outcomes associated with implementation of guidelines²⁴. Tables with details on the quality appraisals are provided in an Appendix.

No studies were excluded from our synthesis purely based on their quality appraisal. However, their quality was considered during the critical assessment of the evidence, when drawing conclusions and drafting recommendations. It is important to note that the quality of the primary studies/evidence included in the selected guidelines was not checked and was instead, taken as reported by the authors of the guidelines.

The quality appraisal was independently performed by one reviewer, after a pilot test was carried out in a limited number of selected studies (n=5).. Three researchers overall were involved in this exercise, one performed the appraisal for *H. pylori*, a second one for coeliac disease and a third one for all EM indications. Results were discussed amongst them in order to harmonise evaluation.²⁵

3.2 Results: Immunohistochemistry in the diagnosis of *H. Pylori*

3.2.1 Introduction

H. pylori is a bacterium known for its link to pathologies such as duodenal and gastric ulcers, chronic gastritis, atrophic gastritis, iron deficiency, mucosa-associated lymphoid tissue (MALT)-type lymphomas of the stomach, and gastric adenocarcinoma.²⁶

It is a common infection that affects up to half of the world's population. The only Belgian study assessing the prevalence of *H. pylori* in adults dates from the 1990s and estimated a prevalence between 32 and 36%.^{27, 28} This appears to be in line with the estimates available for Europe which range from 20 to 40%.²⁹ Nevertheless, the prevalence in Belgium is thought to have declined and be closer to 20% nowadays.⁸

H. pylori can be diagnosed with an invasive procedure (gastroscopy and tissue biopsy), or with non-invasive procedures (tests that do not involve direct tissue biopsy).

When diagnosing *H. pylori* with an invasive procedure, the following tests can be carried out to detect the bacteria:

Rapid Urease Tests (RUT): This test uses the urease activity of *H. pylori* to change the colour of a substrate when tissue biopsy fragments are applied. It is inexpensive and offers high specificity (>95%) and sensitivity (85-95%). However, its sensitivity decreases when only a few *H. pylori* organisms are present. This test does not allow for the morphological assessment of the mucosa, unless a parallel histological analysis is performed.³⁰

Cultures: Cultures can be performed directly from biopsy samples with almost 100% specificity for *H. pylori* infection. However, their sensitivity is lower compared to other assays, and the samples require immediate processing as *H. pylori* organisms are fragile outside their native environment. Like the rapid urease test, cultures do not allow for morphological assessment of the mucosa.³⁰



Molecular Testing: *H. pylori* can be detected through in situ hybridization or polymerase chain reaction (PCR). These methods have high accuracy but they also detect both live and dead organisms and are more expensive, making them less common for routine patient evaluations and more often used to verify antibiotic resistance.³⁰

Histologic examination

Formalin-embedded tissue samples can be examined with H&E staining protocols that, when optimized, can reach sensitivities approaching 100%²⁵. Accuracy can be enhanced further using histochemical or immunohistochemical staining.

Histochemical stains include Wright-Giemsa, "Genta" "Wharting starrey" thiazine alcian yellow and, tholuidine blue.

Immunohistochemical staining is regarded as the gold standard for *H. Pylori* detection, showing nearly 100% sensitivity and specificity.²⁵

When organisms are plentiful, there is little to no advantage to using immunohistochemical compared to histochemical stainings,³¹ but, since the widespread use of PPIs, *H. Pylori* organisms are becoming fewer in number, have a tendency to have a coccoid form, can be found intracellularly and have a different topography than a non-PPI treated infection. All these features contribute to a decrease in the accuracy of H&E in detecting *H. Pylori*.³²

Available non-invasive tests include :

Anti-Helicobacter antibodies: This method involves detecting antibodies formed against *H. pylori* in patients. Serologic tests are highly sensitive (90% to 97%) for *H. pylori* infection. However, these antibodies can persist even after successful eradication of the infection, meaning that serologic positivity does not always indicate a current infection.²⁵

Helicobacter antigen stool assay: This technique uses either polyclonal or monoclonal antibodies against bacterial antigens in an enzymatic immunoassay to detect *H. pylori* in stool samples. These assays are nearly 95% effective in detecting active *H. pylori* infections.³³ Their sensitivity is not significantly affected by the use of PPIs, making them a reliable method of detection, even in patients undergoing such treatments.³⁴

Urease breath test (UBT): Based on the principle that *H. pylori*-infected stomachs exhibit urease activity, this test involves patients ingesting urea labelled with a carbon isotope (either ¹³C or ¹⁴C). The urease produced by *H. pylori* cleaves this labelled urea to produce labelled carbon dioxide, which is then detected in the patient's exhaled breath. The urease breath test is highly sensitive (>95%) and specific (>95%) in detecting active infection,³⁵ although its sensitivity can be decreased in patients treated with PPIs.³⁴

Confronted with a positive test, it is important to treat it and to assess if it has been effectively eradicated, since over time, *H. pylori* has developed resistance to certain antibiotics, making it more challenging to treat.

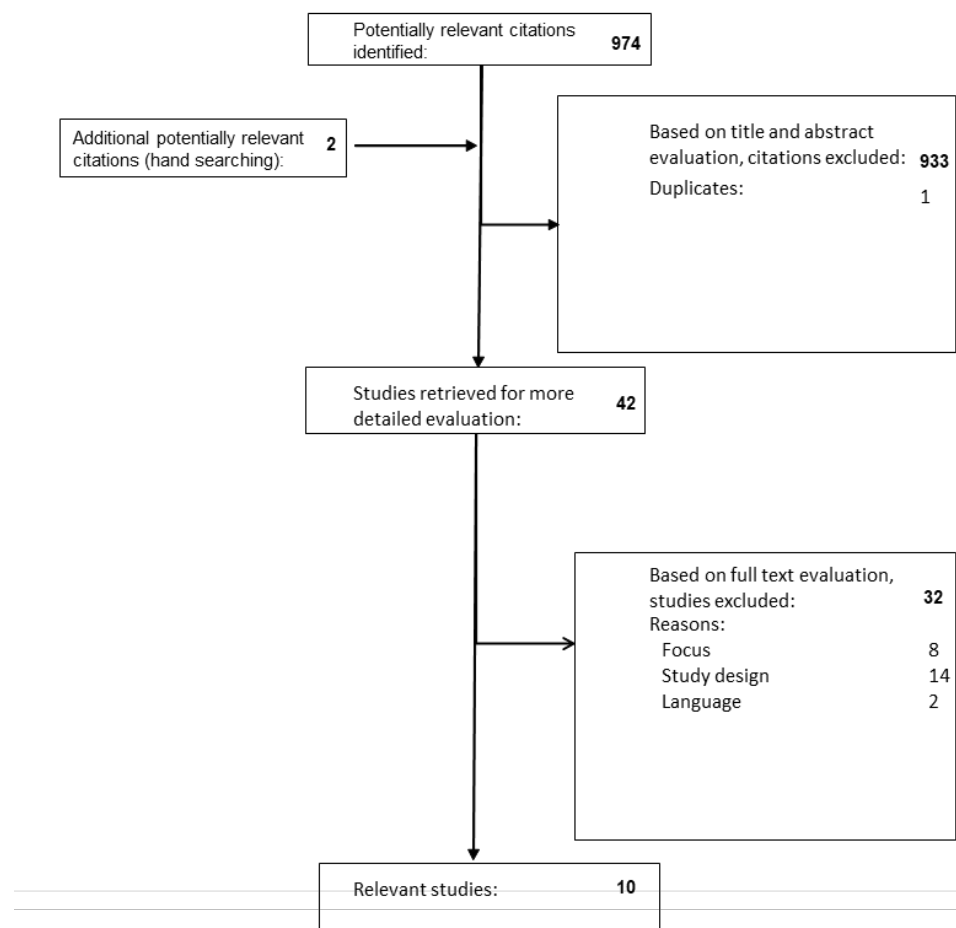
According to the Belgian consensus for *H. pylori* management 2023, "the first line treatment for *H. pylori* recommended in Belgium is either a non-bismuth quadruple therapy (PPI, clarithromycin, amoxicillin, and metronidazole) for 14 days or a quadruple combination with bismuth (PPI, bismuth, tetracycline and metronidazole) for 10 days".⁸ In Belgium, resistance to clarithromycin is estimated to be between 13.5% and 19.8 %. Resistance to nitro-imidazoles varies from 29.7% to 51.6%, as well as resistance to fluoroquinolones from 22.2% to 31.7%. Resistance to amoxicillin and tetracycline remains very low⁸. It is therefore, mandatory to perform a post-eradication *H. pylori* test to detect treatment failure, since in case of two consecutive empirical treatment failures, an antibiotic sensitivity test (AST) is recommended to inform a subsequent targeted treatment.⁸



3.2.2 Guidelines and Systematic Reviews on *H. pylori* Diagnosis

3.2.2.1 Study selection

Figure 16– Flow Chart for study selection – *H. pylori*





Titles and abstracts of 976 studies were screened, from which 42 were selected for full text analysis. Of these, one could not be retrieved, and of the remaining 41, 31 were excluded after full text analysis (see Figure 16).

The ten retrieved papers included nine guidelines on the management of *H. pylori*^{8, 9, 36-42}, and one specifically focused on the use of special stains in the diagnosis of *H. pylori*.²⁵

No relevant SRs were retrieved during this search. This may be due to the limited time horizon of our search. However, non-recent evidence should have been captured via our analysis of clinical guidelines.

3.2.2.2 Quality appraisal

Quality appraisal was restricted to four clinical questions, relevant to our research: who to test for *H. pylori*, how to test for *H. pylori*, how to make a histological diagnosis of *H. pylori* and how to test *H. pylori* eradication. Quality appraisal is not to be intended as necessarily an appraisal of the whole guideline.

Among the nine guidelines retrieved on the clinical management of *H. pylori*, quality appraisal relative to the relevant clinical questions was as follows: one was evaluated as low quality,⁴⁰ two were evaluated as low-intermediate quality,^{8, 37} two were evaluated as intermediate quality,^{38, 42} three were evaluated as intermediate-high quality,^{9, 36, 41} and one was evaluated as high quality.³⁹

The one guideline on the specific use of ancillary stains for the histologic diagnosis of *H. pylori* was evaluated as low quality.

The most common pitfalls hindering the quality of the *H. Pylori* retrieved guidelines were linked to their development process of: lack of reported systematic search of the literature, lack on the reporting of PICOs and search strategies, lack of access to evidence tables, lack of details on the composition of the guideline development group and their potential conflicts of interest and lack of involvement of patients or patients' representatives in the guideline development.

The list of included guidelines and relative overall quality appraisal evaluation, restricted to the four relevant clinical questions is displayed in Table 27.

**Table 27 – List of selected guidelines**

First author, year	Title	Type	Quality appraisal for relevant questions
Chey , 2017	ACG Clinical Guideline: Treatment of Helicobacter pylori Infection	HP management	Intermediate-High
Smith, 2017	The Irish Helicobacter pylori Working Group consensus for the diagnosis and treatment of <i>H. pylori</i> infection in adult patients in Ireland	HP management	Intermediate
Romano, 2022	Management of Helicobacter pylori infection: Guidelines of the Italian Society of Gastroenterology (SIGE) and the Italian Society of Digestive Endoscopy (SIED)	HP management	Intermediate-High
Malfertheiner, 2022	Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report	HP management	Intermediate-High
Katelaris, 2023	Helicobacter pylori World Gastroenterology Organization Global Guideline	HP management	Low
Jones, 2017	Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016)	HP management	High
Georgopoulos, 2020	Hellenic consensus on Helicobacter pylori infection	HP management	Intermediate
Garces-Duran, 2023	Belgian consensus for Helicobacter pylori management 2023	HP management	Low-intermediate
El-Serag , 2018	Houston Consensus Conference on Testing for Helicobacter pylori Infection in the United States	HP management	Low-intermediate
Batts, 2013	Appropriate use of special stains for identifying Helicobacter pylori: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society	IHC use	Low

HP: *H. pylori*



3.2.2.3 Guidelines on *H. pylori* management

We retrieved nine guidelines on the clinical management of *H. pylori*. Eight of these are meant to be used in the general population (explicit mention of adult population or no explicit mention of age group) and one is targeted for the detection and treatment for children and adolescents under the age of 18.³⁹

The methodology behind the development of guidelines mainly follows the same steps: guideline coordinators highlighted domains where recommendations were needed. Experts were requested to do a literature review on such domains and elaborate recommendations supported by the evidence. The recommendations were discussed among experts and finalised via a consensus process (most frequently a Delphi consultation).

Finalised guidelines display evidence grading accompanied by consensus level and in some instances strength of recommendations.

3.2.2.4 Who to test for *H. pylori*

The most frequent explicit indications for *H. pylori* testing are as follows:

Non-investigated dyspepsia is an indication for *H. pylori* testing in all retrieved guidelines^{8, 9, 36-38, 40-43}, except for the guideline regarding children, where it is recommended against diagnostic testing for *H. pylori* infection in children with functional abdominal pain.³⁹

Low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma. This indication is explicitly stated in five of the retrieved guidelines^{8, 9, 36, 37, 40}. In the Maastricht VI/Florence consensus report it is stated that *H. pylori* eradication is the first line treatment for localised low grade MALToma.(Grade A1, 100% agreement).⁹

History of endoscopic resection of early gastric cancer. According to the Maastricht VI/Florence consensus report,⁹ *H. pylori* eradication is considered mandatory in the case of low-grade or high-grade intra-epithelial neoplasia post ablation (Grade A1, 100% agreement). Another three guidelines report explicitly the indication.^{8, 36, 40}

Long-term acetylsalicylic acid (ASA) and long-term Non-Steroidal Anti Inflammatory Drugs (NSAIDs) users. This indication is explicitly stated in four of the guidelines for both indications (ASA and NSAIDs).^{9, 36, 41} In the *H. pylori* World Gastroenterology Organization Global Guideline, the recommendation for *H. pylori* testing is for NSAIDs naïve users, while in case of ASA, it is for patients at high risk of Peptic Ulcer Disease (PUD) or for chronic low dose ASA users if there is a history of GI bleeding.⁴⁰ The Belgian consensus for *H. pylori* management 2023, indicates the recommendation only in the case of NSAIDs users.⁸

Past or present duodenal and/or gastric ulcer (PUD). This indication is explicitly stated in five guidelines,^{8, 36, 37, 39, 40} in another two, the eradication of *H. pylori* is recommended in long term ASA or NSAIDs users with a history of PUD.^{38, 41} It is important to note that PUD is the main indication for *H. pylori* eradication in children (Strong recommendation. Quality of evidence: high. Agreement: 100%).³⁹

Unexplained iron-deficiency anaemia (IDA). This indication is explicitly stated in six guidelines, including the Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents,^{8, 9, 36, 39, 41} although it must be noted that *H. pylori* testing should not be part of the initial investigation of IDA and should only be carried out in the cases of refractory IDA where other causes have been ruled out. In the Hellenic consensus on *H. pylori* infection,³⁸ IDA is not cited as a standalone indication for *H. pylori* testing but as an alarm symptom that should prompt investigation in people with dyspepsia.

Immune thrombocytopenia. This indication is explicitly stated in five of the reported guidelines, including the Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents.^{9, 36, 38-41}

Long-term PPI use. This indication is explicitly stated in four of the guidelines.^{8, 38, 40, 41} In two instances, the indication is aimed at patients suffering from Gastro-Esophageal Reflux Disease (GERD), who need to be on long-term PPIs.^{40, 41}



Family history of gastric cancer. This indication is explicitly stated in six guidelines.^{8, 37, 40, 42} The Irish *H. pylori* Working Group consensus for the diagnosis and treatment of *H. pylori* infection in adult patients in Ireland has history of gastric cancer as the only other indication alongside the general indication “All patients with symptoms related to the upper GI tract”, that is a blanket statement covering symptoms not only related to dyspepsia, but also PUD, gastric cancer and MALT lymphoma.⁴² It needs to be noted that the ACG Clinical Guideline: Treatment of *H. pylori* Infection explicitly recommends against testing asymptomatic individuals with a history of gastric cancer.³⁶

Gastric cancer prevention in communities with high incidence. This indication is explicitly stated in three of the guidelines.^{9, 37, 40} In the Houston Consensus Conference on Testing for *H. pylori* Infection in the United States, it is specified to test first generation immigrants from high prevalence areas and to consider testing in Latino and African-American racial ethnic groups.

Other indications mentioned by the guidelines include Vitamin 12 deficiency,^{9, 41} patients’ wishes,⁴⁰ prior to bariatric surgery⁸ and patients with reflux symptoms if at high risk for *H. pylori*.³⁷

It is important to note that *H. pylori* testing indications of the Belgian consensus for *H. pylori* management 2023,⁸ cover most of the indications above and is therefore, in line with *H. pylori* guidelines concerning adults.

3.2.2.5 Invasive vs non-invasive testing for *H. pylori*

Testing for *H. pylori* can be done invasively, via a gastroscopy, or non-invasively. It is logical that an increase in the indications for invasive testing will increase the use of histochemical and immunohistochemical diagnosis of *H. pylori*.

Not all guidelines cite explicit strategies for deciding when to test invasively and when to test non-invasively, for example the Belgian consensus for *Helicobacter pylori* management 2023 does not position itself on the indications of invasive vs non-invasive testing in adults and states in recommendation 3.1 “*H. pylori* infection can be diagnosed using invasive

(endoscopy with biopsies for rapid urease testing (RUT), histology, culture or molecular diagnosis) and non-invasive methods (serology, urea breath test (UBT) and stool antigen test). Clinical factors and performances of diagnostic tests need to be considered when choosing the appropriate test. Strong recommendation, high quality evidence.”⁸

Most other guidelines though give some indications in this respect. Recommendations are mainly based on the age of the patient and the presence or absence of alarm symptoms, since invasive testing is recommended for individuals with a higher risk of gastric cancer. The age threshold varies among guidelines between 45,^{38, 42} 50,^{9, 41} and 60.³⁶ Invasive testing is recommended in “younger” patients, if alarm symptoms are observed.

Invasive testing is considered mandatory in children,^{8, 39} the Joint ESPGHAN/NASPGHAN Guidelines for the Management of *H. pylori* in Children and Adolescents explicitly advises “against a “test and treat” strategy for *H. pylori* infection in children. GRADE: Strong recommendation. Quality of evidence: low (indirect). Agreement: 100%”.³⁹

3.2.2.6 How to perform gastric sampling

Almost all guidelines recommend at least four biopsies, two from the antrum and two from the corpus; one from the antrum and one from the corpus need to be taken for RUT.^{8, 9, 37, 39-42}

Some guidelines add a recommendation to sample also the incisura angularis, in accordance with the updated Sydney System Biopsy Protocol (USSBP).^{8, 9, 37, 41} Other biopsies may be added according to focal lesions visualised during endoscopy.⁹



3.2.2.7 How to diagnose *H. pylori* with invasive testing

Some guidelines include recommendations on how to diagnose *H. pylori* from gastric biopsies, and of these, most of those propose a combination of RUT and histochemical techniques.^{38, 39, 41, 42}

A notable exception is the Belgian consensus for *H. pylori* management 2023, where in recommendation 3.3, it is stated “*H. pylori* detection by IHC should be used routinely. Strong recommendation, moderate quality evidence”.⁸

In the supporting evidence, it is specified that in practice, IHC is performed in nearly all gastric biopsies in Belgium. This is because clinical information about the patient is sparse or unavailable to the pathologist, and because *H. pylori* can be found even in the absence of gastritis.⁸ This recommendation is in apparent contradiction with the supporting evidence of recommendation 3.1, where it is stated that “Histology is the gold standard for the assessment of *H. pylori*-associated gastritis. To detect *H. pylori*, it is desirable to combine a routine H&E stain with conventional stains such as Giemsa stain”.⁸

3.2.2.8 How to test *H. pylori* eradication

Almost all guidelines recommend a non-invasive test (preferably UBT) for post-eradication *H. pylori* testing, unless there is a reason to perform gastroscopy, in which case, histological techniques should be used.^{9, 36-42}

The Belgian consensus for *Helicobacter pylori* management 2023 does not position itself explicitly on the preferred post-eradication test, stating in recommendation 5.4 “We recommend confirmation of successful eradication testing, especially prior to bypass bariatric surgery. Strong recommendation (100% of agreement), high quality evidence.”⁸

3.2.2.9 Recommendations from the Roger C Haggitt Gastrointestinal Pathology Society

One single guideline dealing specifically with the use of ancillary staining including IHC in the diagnosis of *H. pylori* was retrieved: “Appropriate use of special stains for identifying *Helicobacter pylori*: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society”.²⁵

This guideline was published in 2013, and was evaluated by our research group as having a “low quality” because of the lack of transparency on the methods used to retrieve literature and to establish consensus.

The aim of the guideline is to critically evaluate the literature regarding the utility of ancillary stains for *H. pylori*, and to propose practice guidelines for their use.

The conclusions of the guideline is that most cases of *H. pylori* can be diagnosed, or at least suspected, via an H&E staining alone, since biopsies of infected individuals invariably display inflammation. They recommend IHC if an ancillary staining is required, since histochemical staining such as Giemsa, are of little added value if H&E staining is carefully conducted and reviewed.

Nonetheless, the recommendations are against “reflexive” staining and list precise indications for IHC staining (see Table 28).



Table 28 – Recommendations for the use of Immunohistochemistry (IHC) according to the Rodger C. Haggitt Gastrointestinal Pathology Society for the diagnosis of *H. pylori*. Table modified from Batts et al, 2017²⁵

Morphologic Findings	GIPS Recommendations For Special Stains (IHC)
Normal gastric mucosa	Not indicated
Chemical (reactive) gastropathy	Not indicated if chemical injury is only abnormality. Appropriate if superimposed chronic gastritis is present
Chronic active gastritis	Not indicated if H&E demonstrates organisms. Appropriate if H&E is negative for <i>H. pylori</i> . Low yield if serologic studies are known to be negative.
Chronic inactive gastritis	Not indicated if serologic studies are known to be negative, but probably justified in most other cases. Appropriate if gastroduodenal ulcers are present. Appropriate if gastric MALT-type lymphoma or adenocarcinoma is present. Appropriate if duodenal lymphocytosis is present. Appropriate in patients with prior <i>H. pylori</i> treatment. Appropriate in high-risk demographic areas.
Lymphocytic gastritis	Appropriate
Granulomatous gastritis	Unclear utility; no recommendation at this time
Eosinophilic gastritis	Unclear utility; no recommendation at this time
Isolated chronic active carditis	Appropriate
Isolated chronic inactive carditis	Not indicated, unless gastric biopsies are unavailable and/or serologic studies are positive
Fundic gland polyps	Not indicated
Hyperplastic polyps	Generally not indicated; ancillary stains may be considered if chronic inflammation is present and other biopsies are lacking
Barrett oesophagus	Not indicated
Duodenal biopsies	Not indicated in overwhelming majority of cases



3.2.3 References provided in the online survey

The participants to the online survey (see section 2.2.10) provided excerpts from two references that supported their use of IHC in the diagnosis of *H. pylori*: Arnold's "Atlas of gastrointestinal pathology"¹³, a textbook that pragmatically follows the pathologists' diagnostic process of the interpretation of neoplastic and non-neoplastic GI biopsies, and Lash's "Inflammatory disorders of the stomach",¹⁴ another reference pathology textbook.

3.2.3.1 Atlas of gastrointestinal pathology

In the excerpt provided by the survey participants, the authors highlight how *H. pylori* can be easily spotted on H&E without the use of ancillary stains. IHC should be used in challenging cases, such as treated cases where the organisms are small rounded and rare.¹³ The "Pearls and Pitfalls" section that is reported in the excerpt, reports the indications and contraindications to the use of IHC in *H. pylori* diagnosis as they are seen in "Appropriate use of special stains for identifying *Helicobacter pylori*: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society"²⁵ (see section 3.2.2.4).

3.2.3.2 Inflammatory disorders of the stomach

In the excerpt provided by the survey participants it is highlighted how the frequency of *H. pylori* negative chronic gastritis has increased in the western world due to the effect of antibiotic therapies used to treat other infections, the masking effect due to the use of PPIs, and suboptimal sampling or staining. The use of IHC staining in all biopsies, in the opinion of the authors, should allow the pathologist to detect *H. pylori* both in the case of otherwise *H. pylori* negative chronic gastritis and even in those biopsies where no active inflammation is seen.¹⁴

3.2.3.3 PathologyOutlines.com

Survey participants referred to the "H. Pylori" page of the website "PathologyOutlines.com".¹⁶ Once again the authors referred, for IHC staining indications to "Appropriate use of special stains for identifying *Helicobacter pylori*: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society"²⁵ (see section 3.2.2.4).

3.2.4 Conclusions

In the diagnostic pathway of *H. pylori*, the number of IHC stains performed depend on a cascade of clinical decisions that include: indications to test for *H. pylori*, indications to perform an invasive *H. pylori* testing, the number of biopsies taken per endoscopic session, the preferred method for *H. pylori* eradication testing, and the use of upfront versus reflexive IHC staining.

Guidelines are relatively homogeneous in their recommendations, but in some instances the Belgian consensus for *H. pylori* management 2023⁸ is an exception.

The guidelines considered in this analysis broadly agree on the indications for *H. pylori* testing.^{8, 9, 14, 36-42} Most of them indicate an age threshold for invasive investigation for adults with dyspepsia without alarm signs,^{9, 36, 38, 41, 42} but this is not the case for the Belgian consensus for *H. pylori* management 2023,⁸ where no testing decisional tree is suggested and the choice of the appropriate test is left to the clinician's appreciation.

The number of recommended biopsies is generally considered to be four or five, with the possibility of sampling further focal lesions.^{8, 9, 37, 39-42}

Most guidelines suggest the use of non-invasive methods for *H. pylori* eradication testing, unless endoscopic surveillance is needed for other reasons. The Belgian consensus for *H. pylori* management 2023⁸ does not explicitly position itself on a preferred test method for eradication, although it recommends against the use of RUT, in line with other guidelines.



The majority of guidelines that position themselves on *H. pylori* diagnosis on gastric biopsies, indicate the use of a combination of RUT and histochemical methods as the preferred strategy.^{38, 39, 41, 42} The Belgian consensus for *H. pylori* management 2023⁸ is the only one to explicitly recommend the use of upfront IHC testing for the diagnosis of *H. pylori*, supporting this choice as the most pragmatic course of action, since the reflexive use of IHC staining would be more labour intensive, with IHC being needed anyway in a high number of cases.

Other guidelines that cite explicitly the use of IHC do not advocate its upfront use,^{38, 41, 42} even if the technique is recognised as being the gold standard, since the authors mention it is not considered to be cost-effective.³⁸ Such statement is not backed up by a relevant references, so it remains difficult to interpret, since cost-effectiveness depends on country specific prevalence rates, as well as on the accuracy (i.e. sensitivity and specificity) of the different techniques, and their overall cost. In addition to that, incremental cost-effectiveness would need to be interpreted in the context of country specific willingness-to-pay-thresholds. Whether Georgopoulos et al. simply refer to the fact that other techniques are less costly remains unclear, since a detailed review focused on the cost-effectiveness of the difference techniques was out of scope of our research, and only when a specific mention was made on that regard in the included guidelines or SRs, this was briefly reported, for completeness.

The excerpts from the textbooks and the web site provided by the survey participants feed into the debate on whether or not upfront IHC staining is recommended. On the one hand, in the Atlas of gastrointestinal pathology¹³ and in the PathologyOutlines.com web site,¹⁶ the authors do not advocate for upfront IHC staining and rely on the recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society.²⁵ On the other hand, in the textbook "Inflammatory disorders of the stomach",¹⁴ authors advocate for upfront IHC staining, in order to enhance diagnostic accuracy, especially in cases of *H. pylori* negative chronic gastritis and in biopsies where no active inflammation is observed.

This debate is present also within the Belgian consensus for *H. pylori* management 2023 itself.⁸ In their recommendation 3.3, it is stated that IHC should be used routinely for the diagnosis of *H. pylori*. However, in the supporting evidence they discuss for a further recommendation (i.e. recommendation 3.1), it is stated that a combination of H&E and Giemsa staining should be used.

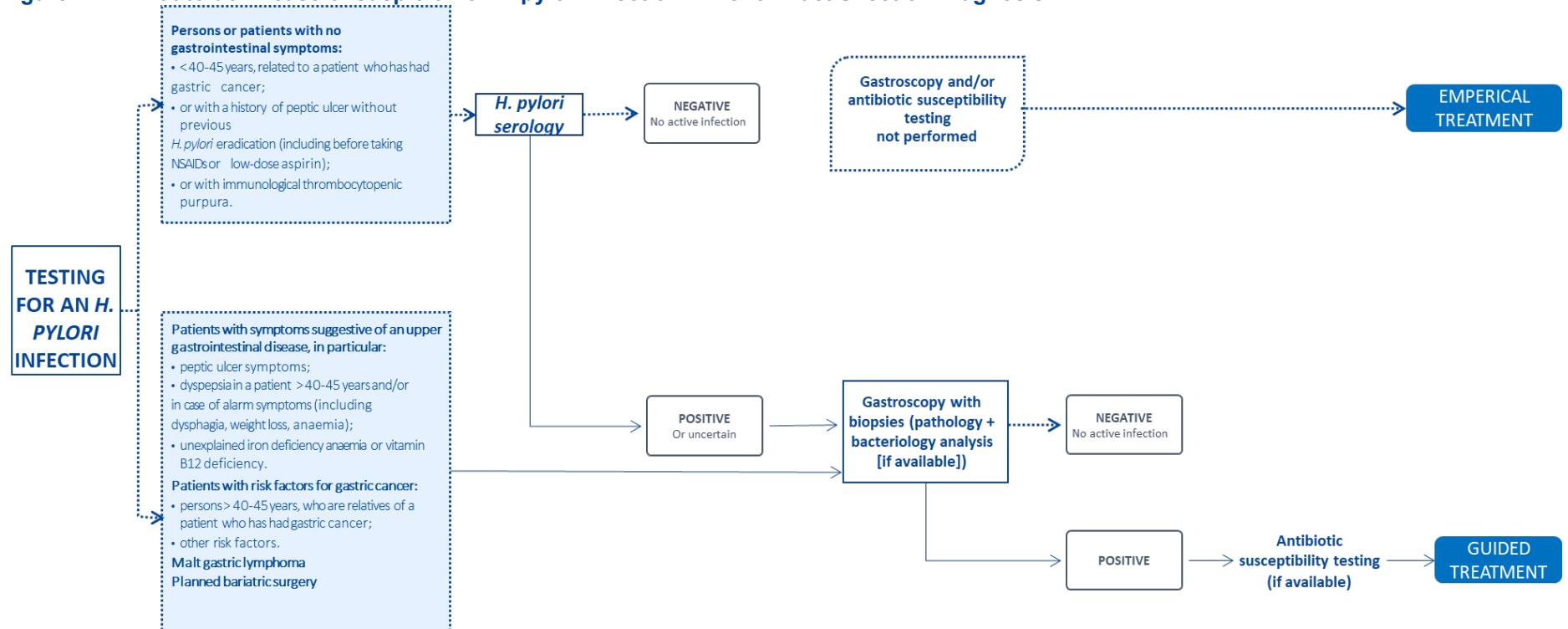
3.2.5 Evidence from International Evaluations

3.2.5.1 Fact sheets on the diagnosis and treatment of *H. pylori* by the French National Authority for Health and the National Professional Council for Hepato-Gastroenterology

In May 2017, the French National Authority for Health (Haute Autorité de Santé - HAS),^k in collaboration with the French National Professional Council of Hepato-Gastroenterology (CNP-HGE) published a guideline on the appropriateness of biological acts and prescriptions in adult patients infected with *H. pylori*.⁴⁴ As part of this work, two fact sheets on the diagnosis and treatment of *H. pylori* infection in adults were drafted, to support health care professionals in making decisions about adult patients infected with *H. pylori*.⁴⁵ The first of these fact sheets, is of particular interest to our research and thus, its content is briefly described here.

In order to develop these guides, an analysis of administrative data was performed to assess to what extent, clinical practice in France deviated from European recommendations.⁹ Their data analysis appeared to indicate a deviation in certain cases between clinical practice and international recommendations. An effort was therefore made to develop the fact sheets for practitioners in order to illustrate what the flow in decision making should be. Figure 17 shows that flow.

^k https://www.has-sante.fr/jcms/pprd_2986129/en/home

Figure 17 – What to do in case of suspicion of *H. pylori* infection – French Fact Sheet on Diagnosis¹¹ Available at : https://www.has-sante.fr/upload/docs/application/pdf/2017-09/helicobacter_pylori_-_tool_for_appropriate_diagnosis.pdf



It is important to highlight that at the time these fact sheets were published (2017), the only non-invasive diagnostic test listed in the French nomenclature (and therefore reimbursed) was serology. This is why only serological tests appear in their algorithm. Nevertheless, the fact sheet also mentions the good performance for diagnosis and for eradication control of the UBT and the stool antigen tests.

Following this, an evaluation of the various non-invasive tests for detection of *H. pylori* in asymptomatic subjects, as well as for checking eradication after a positive *H. pylori* infection, was pursued by HAS.⁴⁶ This was based on a review of the literature and offered a favourable advice for the inclusion in the nomenclature of the UBT, the stool antigen test and the gene amplification techniques (the latter specifically for the identification of *H. pylori* coupled with the search for clarithromycin resistance mutations). These tests are reimbursed in France since September 2022.

The algorithms and the research undertaken focused on offering advice on when invasive or non-invasive tests should be recommended, which would in turn have an impact on the target population on which IHC could be performed, but did not evaluate which tests specifically should be used, after a gastroscopy. Thus, no specific evaluation of IHC tests for the diagnosis of *H. pylori* was carried out as part of their research.

The mean expenditure per IHC examination in France in 2022 was €54, of which €43 was reimbursed and €11 were patient out of pocket expenses.

3.2.5.2 Report on stomach complaints by the National Health Care Institute of the Netherlands (ZIN)

In July 2021, an improvement report on stomach complaints was published by the National Health Care Institute of the Netherlands (Zorginstituut Nederland - ZIN).^m This research was carried out in collaboration with relevant organizations of patients, healthcare professionals and health insurers. It consisted of an analysis of guidelines (mainly limited to Dutch guidelines, but also guidelines by the National Institute of Health and Clinical Excellence – NICEⁿ) to assess the consistency of their conclusions, their

quality and clarity, followed by a data analysis to evaluate to what extent clinical practice in the Netherlands followed the guidelines identified. The focus was on stomach complaints linked to gastroesophageal reflux disease and (functional) peptic ulcers. On the basis of their analysis, improvements were proposed in the following areas:

1. Need for updating the existing guidelines and improving patient information;
2. Reducing unnecessary gastroscopies;
3. Reducing overtreatment with PPIs in adults;
4. Reducing overtreatment with PPIs in infants;
5. Improving the quality of anti-reflux surgery.

Points 1 (need to update the existing guidelines) and 2 (reducing unnecessary gastroscopies) were thought to be of interest for our study.

Given the specific focus of our report, the description of ZIN's research here presented, is limited to our area of interest: i.e. recommendations for invasive testing, and for diagnosis of *H. pylori*, and more specifically, a description of what the guidelines included in the review by ZIN, recommend on that regard.

Their methods consisted of a search for Dutch and international guidelines. For the latter, only the most relevant guidelines for the Dutch context were selected, and such selection was done in consultation with stakeholders.

Their research concluded that the recommendations captured in the guidelines retained⁴⁷⁻⁵⁰ were not always clear and some inconsistencies existed. Moreover, major differences in their quality (according to the AGREE-II tool) were highlighted, with two being appraised by the authors of ZIN's report as having high quality,^{48, 49} a further presenting moderate quality⁴⁷ and the remaining considered of low quality.⁵⁰

^m <https://english.zorginstituutnederland.nl/>

ⁿ <https://www.nice.org.uk/>



Some of these guidelines were categorised as outdated^{48, 50} or their conclusions were considered not well supported by the existing evidence, which in turn, (partly) explained the inconsistencies identified in their recommendations.

The main recommendations captured on the use of non-invasive testing for *H. pylori* are summarised in Table 29, while Table 30 looks at the indications in which gastroscopies are to be performed.

Table 29 – Guidelines on non-invasive testing for *H. pylori* diagnosis (ZIN 2021⁵¹)

NHG	CBO	NVMDL	NICE
Primary investigation	Primary examination	N/A	Primary examination
Stool test or a C13 breath test is preferred over serological testing	Serological examination preferable to stool test or UBT		C13 UBT, stool test or serological examination
Eradication control	Eradication control		Eradication control
See primary research	UBT preferred to serological testing		UBT

Substantiation/considerations

Guidelines express different preferences based on available evidence about the reliability of the tests and other (practical) considerations at the time of guideline development.

Regarding the choice of non-invasive test for *H. pylori*, the recommendations made in the (moderate quality) NHG-Standaard maagklachten guideline for GPs⁴⁷ privilege monoclonal stool testing or a C13 breath test, as a first step. A point is made regarding the fact that the stool test is cheaper in the Netherlands, while UBT is not always available. On the other hand, the C14 breath test is not recommended, primarily due to its radioactivity and higher cost. Similarly, serological testing (ELISA) is not recommended, because of its lack of reliability.

The (good quality) guidance by NICE⁴⁹ on non-invasive testing for *H. pylori* recommends the use of carbon-13 UBT, a stool antigen test, or laboratory-based serology (although the latter only when its performance has been locally validated). They specifically recommend not to use office-based serological tests for *H. pylori* because of their inadequate performance. Carbon-13 UBT is the only test they recommend to check eradication, due to the fact that they consider the existing evidence for stool eradication testing insufficient to support such use.

The guideline by CBO⁴⁸ was ranked by the authors of the ZIN report as having a good quality. However, it appears to contradict the previously described guidelines, recommending the serology tests over the stool antigen test and the UBT. Nevertheless, it is important to highlight that this is an older guideline.

Regarding invasive diagnostic testing, the two more recent guidelines included in ZIN's report^{47, 49} recommend a gastroscopy in the presence of alarm symptoms (i.e. unintentional weight loss; persistent vomiting; vomiting blood; or black stools), or for controlling gastric ulcers. In addition to this, the NHG guidelines also recommend to test any individuals aged over 40, with a first relative having a history of gastric cancer.

Both guidelines also mention that consideration for a gastroscopy could be made in "older" patients (aged over 50 in the NHG and over 55 in NICE's guidelines), with *H. pylori* and a lack of response to antacids.


Table 30 – Indications for a gastroscopy in stomach complaints (ZIN 202151)

NHG	CBO	NVMDL	NICE
Absolut indications - Alarm symptoms - Control of gastric ulcer - Age >40 years with 1st-degree relative with gastric cancer	Alarm symptoms - Gastric ulcer control - Control of reflux oesophagitis, grade C/D	- Usually with GI complaints that do not respond to PPIs - No repeated gastroscopy after recent gastroscopy	Alarm symptoms Control of gastric ulcer
Relative indications - Persistent stomach upset - Aged >50 years on antacids and with <i>H. pylori</i> diagnosis, especially in older patients, males or those presenting risk factors for esophagus or stomach cancer - Need for diagnostic certainty - No gastroscopy in patients aged <50 without alarm symptoms	To be considered - Persistent gastric complaints: especially in elderly patients and with a strong need for diagnostic certainty (before or after treatment with PPIs or <i>H. pylori</i> diagnosis) - Gastroscopy no longer of value in younger patients with persistent complaints after treatment with PPIs and negative <i>H. pylori</i> test		To consider - Persistent gastric complaints at age >55 years after treatment with PPIs and <i>H. pylori</i> diagnostics - Exclude Barrett's oesophagus in case of demonstrated reflux disease, depending on patient preferences and risk factors - No repeated endoscopy in the absence of alarm symptoms

Considerations

NHG, CBO and NICE recommend a gastroscopy in case of alarm symptoms based on consensus. NHG, CBO and NICE base the indication of gastroscopy at an older age for persistent gastric complaints on the prevalence figures of oesophageal and gastric cancer. NHG and CBO rely on consensus and limited evidence for indication certainty. NVMDL does not provide substantiation.

Similarly to the previously described fact sheets developed by HAS, the guidelines described in the report by ZIN, do not offer any statement on the type of test that should be performed following a gastroscopy. Their focus is once more, on when invasive or non-invasive testing should be used, in the broader context of GI complaints.

H. pylori diagnostics is reimbursed in the Netherlands. However, these costs are charged to the "patient's own risk". Patients pay €385 per year of "own risk" in the Netherlands.



Conclusion – Guidelines and SRs on *H. pylori* diagnosis

- Our analysis showed that *H. Pylori* is the most common non-oncologic diagnostic indication for IHC.
- In the diagnostic pathway of *H. pylori*, the number of IHC stains performed depend on a cascade of clinical decisions that include: indications to test for *H. pylori*, indications to perform an invasive *H. pylori* testing, the number of biopsies taken per endoscopic session, the preferred method for *H. pylori* eradication testing, and the use of upfront versus reflexive IHC staining.
- Most guidelines offer an age threshold for performing invasive testing for *H. pylori* in the absence of alarm signs, but Belgian guidelines leave the choice open to the physician.
- Among those guidelines that give recommendations on the histological diagnosis of *H. pylori*, most do not recommend upfront IHC testing, Belgian guidelines are an exception in this respect, with an explicit recommendation on the upfront use of IHC, but the discussion of a previous recommendation contradicts this statement.
- Most guidelines recommend UBT to be used for post-eradication testing, but Belgian guidelines leave the choice open to the physician.
- HAS and ZIN guidelines focus on whether invasive or non-invasive testing should be used (in *H. Pylori* testing in the case of HAS and in the context of GI complaints, in the case of ZIN).

3.3 Results: Immunohistochemistry in the diagnosis of coeliac disease

3.3.1 Introduction

Coeliac disease is a chronic, serious, multiorgan autoimmune disease, triggered by the ingestion of the protein gluten. It affects the small intestine, interfering with the absorption of nutrients from food, in genetically predisposed persons.

In adults, the most classical symptoms include chronic diarrhoea, weight loss and abdominal pain. Nevertheless, symptoms experienced by coeliac disease patients vary greatly with some patients presenting minimal or no symptoms, and others presenting more uncommon symptoms, such as anaemia, mouth ulcers, fatigue, etc. Children are more likely to experience digestive symptoms. Their inability to efficiently absorb nutrients often causes weight loss, growth retardation, anaemia, or delayed puberty. The lack of specificity in symptoms, makes diagnosis challenging.

Some population groups are at a higher risk of presenting the disease. These include individuals having first-line relatives with CD, or those suffering from certain diseases, such as thyroid, or liver disease.⁵²

Diagnosis is made by a combination of serological tests, clinical history, histology of duodenal biopsies and exposure to a gluten free diet to check response. The only treatment is a life-long gluten-free diet. Unchanged or recurrent symptoms under gluten-free diet may indicate refractory celiac disease,

The prevalence of coeliac disease has significantly increased over the past 50 years, and is estimated to be around 1% of the overall population with a substantial increase of its incidence, partly due to both better diagnostic tools and approaches and a more thorough screening of high-risk individuals.^{52, 53}



A systematic review published in 2020, showed that in the 21st century, the pooled female incidence of coeliac disease was 17.4/100 000 person/years, compared to 7.8 in males. The incidence in children was 21.3 per 100 000 person-years (95% CI: 15.9; 26.7), versus 12.9 in adults. Interestingly, the study showed the incidence of coeliac disease to have been annually increasing by as much as 7.5% over the past several decades.⁵⁴

3.3.2 *Guidelines and systematic reviews on coeliac disease diagnosis*

3.3.2.1 *Study selection*

Our search for guidelines and SRs for coeliac disease (see appendix for details on the search strategy and PICO) resulted in 1104 hits overall, 809 after duplicates were removed. To these, an additional reference found by hand searching was added. A total of 791 articles were excluded on the basis of title and abstract, leaving 18 potentially relevant guidelines, for which full texts were retrieved for consideration. Four were excluded because they were published before 2013.⁵⁵⁻⁵⁸ A further two were excluded because they covered guidelines that had been updated.⁵⁹⁻⁶¹ Under those circumstances, only the most up to date were kept for our review. Two articles were excluded because they consisted of descriptions of a guideline already included in our selection.^{62, 63} One more presented a wrong focus, looking at the sensitivity and specificity of certain symptoms, diseases or genetic factors, to conclude what populations should be screened for CD.⁵²

From the remaining nine, one consisted of a review of guidelines and was kept for reference checking purposes only,¹⁰ leaving us with a total of eight studies to be considered. Figure 18 offers a flow chart with the screening and selection process.

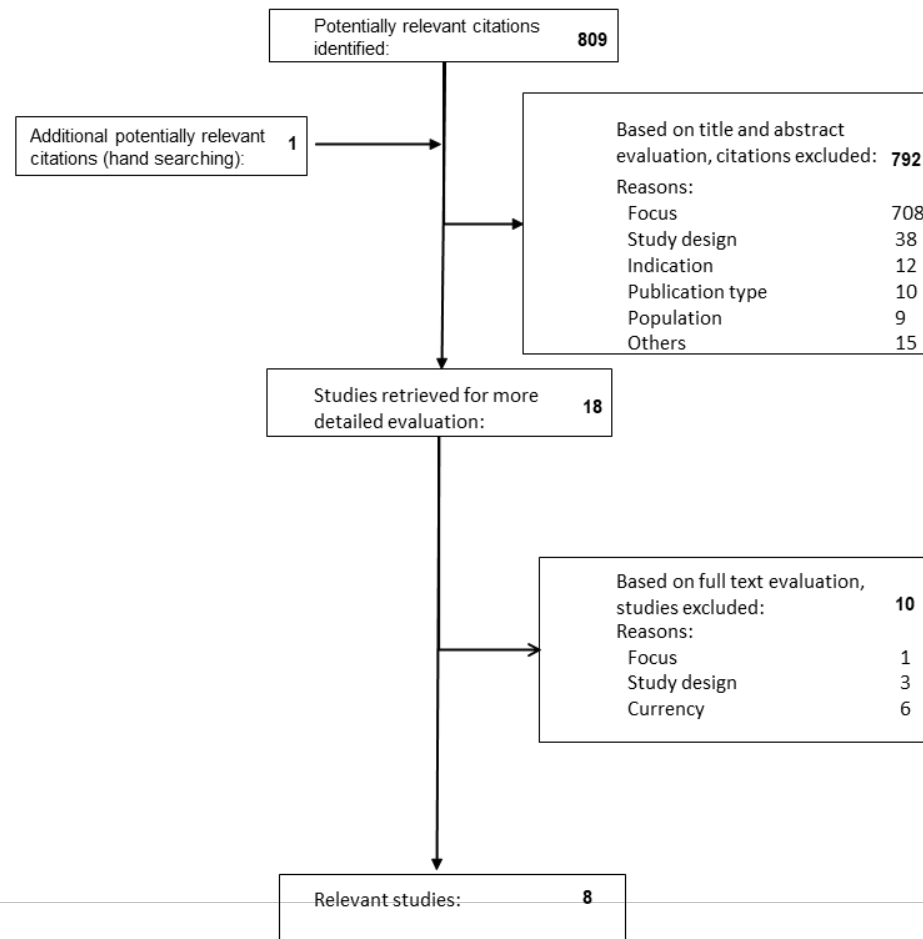
**Figure 18 – Flow Chart for study selection – Coeliac Disease**



Table 31 offers an overview of the included studies.

Table 31 – Characteristics of included studies

Characteristics of included studies	American College of Gastroenterology (ACG) ⁶⁴	Italian Societies of Gastroenterology (ISG)	European Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)*	European Society for the Study of Coeliac Disease (ECD)	World Gastroenterology Organisation (WGO)	National Institute of Health and Clinical Excellence (NICE)	German Society for Gastroenterology, Digestive and Metabolic Diseases (DGVS) and German Celiac Disease Society (DZG)	British Society of Gastroenterology (BSG)
Author/Yr	Rubio-Tapia/2023	Zingone/2022	Husby/2020	Al-Toma/2019	Bai/2017	Downey/2015	Felber/2014	Ludvigsson 2014
Geographical scope	USA	Italy	Europe	Europe	International (World gastro organisation)	UK	Germany	UK
Method use	Review and grading of existing evidence	Systematic review and grading of existing evidence	Systematic review and grading of evidence	Review and grading of evidence	Not detailed - based on evidence and expert consensus. Upd. of 2013 guideline	Systematic reviews and cost considerations. Grading of evidence performed	Systematic review and Delphi panel discussions	Review and grading of evidence, and online surveys
Search period	Not specified but articles published in 2021 included	Jan 1985 - Jan 2022,	2000 - 2016	Jan 1990 to Aug 2018	Not specified	Up to Dec 2014	Up to Nov 2012	1900 to 2012
Population	Coeliac disease in children and adults	Coeliac patients and dermatitis herpetiformis in children and adults	Coeliac disease in children and adolescents	Coeliac disease and other gluten-related disorders in children and adults	Adults and children with Coeliac disease	Coeliac disease in children and adults	Coeliac disease, wheat allergy and wheat sensitivity in adults and children	Coeliac disease in adults
Quality evaluation (Y/N)	Y	Y	Y	Y	N	Y	Unclear	Y



Tool used for quality assessment (eg. GRADE)	GRADE	GRADE	QUADAS - 2 (RoB) and GRADE	GRADE	NA	GRADE	NA	Oxford Centre for Evidence-based Medicine levels of evidence
Overall quality of guideline (according to AGREE GRS tool)	Low-Intermediate quality	High	Intermediate-High	Intermediate-High	Low	High	Intermediate	Low-Intermediate

Four of the eight guidelines identified were published in 2019 or later, which highlights the importance that the topic has gained in recent years. Six had a European geographical scope, with two European guidelines, one focusing specifically on the paediatric population, a further two British, one German and one Italian. Recent USA guidelines were also identified and included in this review.

All guidelines were based on reviews of the literature, and expert discussions and/or surveys. Regarding the reviews of the available evidence, these were not systematic in all cases, with some guidelines presenting comprehensive reviews. The methods were often not detailed and instead a general description was provided.

One guideline focused in the paediatric population,¹² while a further was limited to adults.⁶⁵ The remaining covered both children and adults.

All but two guidelines^{66, 67} performed an evaluation of the quality of the evidence, and most used GRADE for that purpose.

The results presented in Table 32 offer an overview of the recommendations included in these guidelines that could have an impact on the use of IHC in this field. They therefore, do not give an exhaustive overview of all their recommendations, given the scope of our review.

Thus, the focus was on three aspects: first, who should be tested for CD, second, from those, who should be referred for an endoscopy and finally, are there any specific recommendations or any mention of when IHC stains (i.e. CD3 and CD8) should be used?

Before describing the results of these guidelines, it is important to highlight that, their recommendations of interest to our research appear to be highly consistent, and that differences were more in terms of the strength of the recommendation or the quality of the evidence given by the different expert groups.

Screening the general population is not recommended.^{64, 65, 67, 68} Instead, all guidelines recommend to test patients presenting with symptoms, signs or laboratory evidence suggestive of malabsorption (e.g. Diarrhoea with features of malabsorption, iron deficiency, anaemia in absence of other causes, recurrent nausea, vomiting, unexplained weight loss, failure-to-thrive, growth/ short stature or delayed puberty in children, etc). In general, this was a strong recommendation, based on high-level of evidence.



Nevertheless, given the variety of possible presentations, as well as the higher risk of presenting this disease in certain patients groups, other common targets for testing included in the guidelines were first-degree family members of coeliac disease diagnosed patients, or patients with specific conditions, more prone to developing coeliac disease, such as:

- Type 1 diabetes,
- Unexplained elevation of serum aminotransferase levels,
- Selective IgA deficiency,
- Autoimmune thyroid disease,
- Down syndrome , Turner syndrome or Williams syndrome,
- Dermatitis herpetiformis, psoriasis or other skin disorders,
- Chronic fatigue syndrome or fibromyalgia,
- etc.

For adults, the first step in the clinical pathway to diagnosis is serologic testing. In a second step, all patients with positive results should also undergo endoscopy for confirmation. Under certain specific conditions, children may avoid the second step (i.e. the endoscopy) even in the presence of a positive blood test (see Table 32 for more detail).

All guidelines but one⁶⁶ recommend to restrict the use of endoscopies to patients with a prior positive serologic test. Bai et al. are the exception and recommend to perform an endoscopy and duodenal biopsy, even if coeliac disease serology is negative, in the following cases:

- Chronic (non-bloody) diarrhoea
- Diarrhoea with features of malabsorption, especially weight loss
- Iron deficiency anaemia in absence of other causes
- GI symptoms with a family history of CD

- GI symptoms in patient with autoimmune disease or IgA deficiency
- Failure to thrive in children
- Skin biopsy-proven DH
- Patient with video capsule findings suggestive for villous atrophy
- And unexplained high output ileo-(colo-)stomy.

IHC staining is mentioned for equivocal cases in four guidelines.^{53, 65, 67, 68}

In addition to this, all guidelines that mentioned the type of tests or methods that should be used for diagnosing coeliac disease,^{53, 64, 65, 67, 68} highlight the value of both IHC and flow cytometry in patients with suspected RCD, with two of these studies also mentioning T-cell clonality through T-cell receptor PCR testing as a further option.^{64, 65} No clear preference between IHC or flow cytometry are reported, while one guideline specified that most often, at least two testing methods are used in these more challenging cases.⁶⁵ IHC is recognised as a facilitator for the classification of RCD in type I or type II, having a direct impact on the therapeutic approach and prognosis of RCD.



Table 32 – Main recommendations on coeliac disease diagnosis

Recommendations	Testing to be considered in symptomatic patients (e.g. diarrhoea, weight loss, abdominal pain ...) or high risk groups (eg. 1st-line relatives with CD, thyroid disease, liver disease...)	Serological tests should be used as 1st step (TTG-IgA + total IgA value) in children and adults with normal serum IgA	In patients with IgA deficiency, an IgG based test should be used as a 2nd step	Adults and children with + serological results should be referred for an endoscopy	In children under precise conditions (high level TTH IgA (>10x upper limit of normal) and + EMA in a 2nd blood sample) diagnosis may be completed without endoscopy (if parents agree)	Is IHC (CD3 or CD8) use mentioned in guideline?	Are CD3 or CD8 stains recommended for diagnosis of CD?
American College of Gastroenterology (ACG)	✓ (Strong - low quality)	✓ (Strong - moderate quality)	✓ (Strong - moderate quality)	✓ (Strong - moderate quality)	✓ (Conditional recommendation - moderate quality)	YES	Recommended methods for classification of RCD: IHC, flow cytometry or T-cell clonality through T-cell receptor PCR (Strong- moderate quality for need to classify RCD)
Italian Societies of Gastroenterology (ISG)	✓ (Strong - high quality)	✓ (Strong - high quality)	✓ (Strong - moderate quality)	✓ (Strong - high quality)	✓ (Strong - high quality)	YES	Pathology report should include % of IELs, using CD3 IHC when in doubt. Flow cytometry or IHC are both recommended in patients with suspected RCD but flow cytometry considered superior (Strong - moderate quality to refer suspected RCD patients to a specialised 3ary center for further exploration)
European Society of Paediatric Gastroenterology, Hepatology and	Recommend to test symptomatic children and adolescents (weak)	✓ (Strong - high quality)	✓ (Strong - moderate quality)	✓ (Strong - NA)	✓ (Strong - moderate quality)	NO	NA



Nutrition (ESPGHAN)*	conditional - low-moderate quality)						
European Society for the Study of Coeliac Disease (ECD)	√ (Strong/conditional** - high quality)	√ (Strong - high quality)	√ (Strong - moderate quality)	√ (Strong - high quality)	√ (Conditional - moderate quality)	YES	Pathology report should include % of IELs. IHC for CD3 should be used for equivocal cases. IHC helpful to highlight the distribution pattern for IELs. IHC and flow cytometry both recommended to diagnose RCD (NA)
World Gastroenterology Organisation (WGO)	√ (NA)	√ (NA)	√ (NA)	√ (NA)	Not limited to children: "In certain situations..." but does mention the ESPGHAN guidelines as an example (NA)	Mentions that RCD type II is characterised by clonal expansion of intraepithelial lymphocytes and an aberrant phenotype lacking surface CD3, CD8, and T-cell receptors	Does not specify how, or when IHC stains should be used
National Institute of Health and Clinical Excellence (NICE)	√ (No strength given - very low quality)	√ (No strength given - high-moderate quality)	√ (No strength given. (high-moderate quality)	√ for adults. Children should be referred for further investigation and consideration of a biopsy (No strength given - high-moderate quality)	√ But no specific mention of high level TTH IgA (NA)	NO	NA



German Society for Gastroenterology, Digestive and Metabolic Diseases (DGVs) and German Celiac Disease Society (DZG)	√ (Strong)	EmA-IgA Ab could be used instead of TTG-IgA also in combination with total IgA values (Strong)	√ (Strong)	√ and later a response to a GFD would be needed. The three are necessary to confirm diagnosis (Strong)	Detection of HLA-DQ2 or -DQ8 AND disappearance or reduction of symptoms under a GFD should also be seen (Open recommendation)	YES	IHC can be helpful in equivocal cases (to assess the distribution of IEL). Additional molecular and IHC examinations (CD3, CD8) should be carried out if RCD is suspected.
British Society of Gastroenterology (BSG)	√ (NA)	Combination of IgG-DGP and IgA-TTG considered most sensitive (NA)	√ (NA)	√ (Moderate - category II or III evidence)	NA	YES	Evaluation of IELs (with IHC CD3 in equivocal cases) should be included in pathology reports. Methods to differentiate RCD I and RCD II, include genetic analysis of T-cell receptor clonality, IHC and flow cytometry. Often more than one is used. Relative contribution of each of these unclear. (NA)

* Limited to children and adolescents, ** for asymptomatic first degree family members of a coeliac disease patient

3.3.2.2 Quality appraisal

Quality appraisal was restricted to four clinical questions considered relevant to our research: who to test for coeliac disease, by what means should the target population be tested (invasive versus non-invasive testing), what tests should be used and in what order. The quality appraisal here presented is therefore not intended to offer an appraisal of the whole guideline.

Four out of the eight guidelines included in this review were considered of high or intermediate-high quality.^{53, 68-70} A further was of intermediate quality⁶⁷ and the remaining three presented either a low-intermediate^{64, 65} or a low⁶⁶ quality.

Most of the recommendations previously presented were based on moderate-high evidence (as judged by the authors of the guidelines).

The mentions made specifically on IHC were not “formal” recommendations as such and thus, no quality or strength were attached to them.

The most common weaknesses presented in the guidelines included in this review were similar to those already identified in the review of guidelines for *H. pylori* and included: a general lack of access to the searches carried out or to the PICOs used. Although references were given, no evidence tables were provided or summarised. In some cases the guideline development process was not explained in detail, so reproducibility of the process was low.



The overall quality of the included guidelines is shown in Table 31, while details on the quality assessment are presented in an appendix.

3.3.1 *References provided in the online survey*

The participants to our online survey provided one further reference for consideration in our review.¹⁷ The study, published in 2023 consisted of a descriptive paper, offering an overview on the perspective of pathologists regarding the correct methodological approach for the diagnosis of coeliac disease. Because of its descriptive nature, no quality appraisal could be performed on it.

The article covered all different processes involved in the diagnosis of coeliac disease, from biopsy sampling, orientation, processing, staining and histopathological classification in order to avoid, or minimise errors in interpretation of results and final diagnosis, as well as to limit variations in practices. Nevertheless, given the focus of our research, only the conclusions offered by the authors on staining are summarised here. Villanacci et al.¹⁷ state that while one H&E stained section is enough for a full morphological assessment, IHC with anti-CD3 is considered to be useful to provide a correct count of IEL, particularly in the initial forms of coeliac disease, while CD8 IHC is mentioned to be an option if wanting to exclude RCD, specifically in elderly patients. This appears to be in line with those guidelines previously reviewed and summarised in Table 32.

Conclusion – Guidelines and SRs on coeliac disease diagnosis

- Coeliac disease has a prevalence of around 1% and its incidence has increased significantly in the last decades, mainly due to better diagnosis and screening.
- Our data analysis shows the diagnosis of coeliac disease to be an important indication for IHC use in Belgium, with CD3 and CD8 stains accounting for approximately 14.2% of the total IHC stains performed in GI biopsies in the year 2019.

- The existing international guidelines on the diagnosis of coeliac diseases do not offer specific clear recommendations on what type of test should be performed, and focus instead on when patients should be tested and by what means (serology or endoscopy).
- Only symptomatic patients (or patients at high-risk of coeliac disease) should be tested.
- First line testing should consist of a serologic test (TTG-IgA + total IgA value).
- All adult patients with a positive result should be referred for gastroscopy, while children under certain specific circumstances, may be diagnosed based on non-invasive testing methods only.
- IHC is specifically mentioned and recognised as a valuable test for equivocal cases, as well as for cases in which refractory coeliac disease is suspected.

3.4 **A review of the literature on Electron Microscopy – Focus on key indications**

EM is a powerful and technically demanding imaging technique, which uses electron beams instead of light to achieve higher resolution in visualising ultrastructural details of biological specimens. In clinical diagnostics, EM is valuable for examining tissues at a cellular and subcellular level, aiding in the identification of structural abnormalities which are sometimes crucial for accurate disease diagnosis.

As described in Section 2.3.2, according to NIHDI data, the four most common tissue samples that were analysed for diagnosis with EM in Belgium were kidney (mainly for glomerular diseases), skin (for Ehlers Danlos Syndrome), ENT (for Primary Ciliary Dyskinesia), and muscle (for myopathies). Furthermore, the responses to our survey by Belgian laboratories carrying out EM confirmed these four tissues and indications as the most frequent (particularly kidney, muscle, and cilia). We therefore chose to focus on these four (groups of) conditions and searched for evidence as to the appropriateness of EM as a diagnostic tool.



3.4.1 Evidence for kidney diseases:

According to the analysed data in Section 2.3, kidney biopsies are the tissue type most commonly examined with EM. Renal biopsies are generally carried out in chronic kidney disease (CKD) that cannot be fully diagnosed through non-invasive tests (e.g. serology, urinalysis, imaging) alone and in which biopsy results are expected to guide or modify treatment. Biopsies can concern both native and transplanted kidneys, and sometimes must be carried out more than once, when re-evaluation is necessary.⁷¹

The frequency of chronic kidney disease is reported to be on the rise. While diabetes and hypertension are the primary causes for CKD, chronic renal replacement therapy due to glomerular diseases appears to be increasing. In recent years, various non-invasive methods have been proposed for the early detection of renal damage (e.g. 'omics' techniques).⁷² However, the impact of these biomarkers on patient management and long-term outcomes still awaits validation in routine clinical practice.⁷²

Renal biopsy therefore remains the definitive diagnostic standard in patients with renal parenchymal diseases,⁷³ despite the lack of consensus regarding the appropriate indications and clinical utility of this procedure.⁷² Consequently, the decision to perform a renal biopsy is often based on individual opinions and/or the policies of specific healthcare centres.⁷²

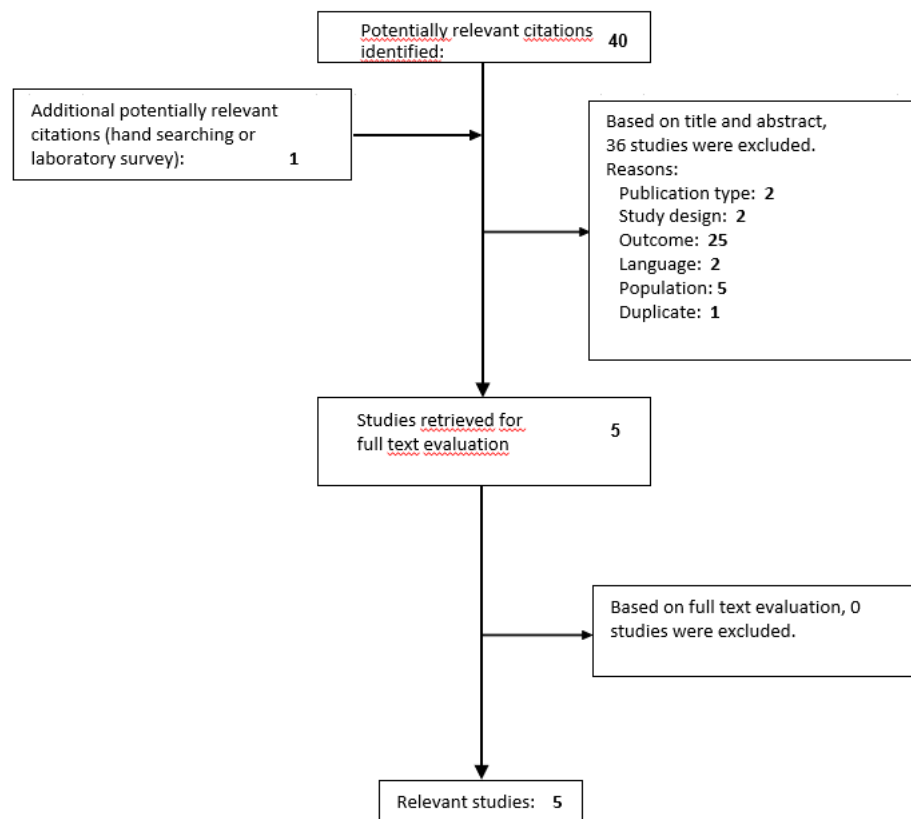
We carried out a literature review to identify clinical guidelines and/or reviews that provide evidence on the most appropriate diagnostic techniques for renal pathology. While we retrieved some articles on different pathologies, we certainly did not identify all indications for renal biopsy, and therefore, potentially, for EM diagnosis. The absence of retrieved guidelines or systematic reviews for other kidney conditions should therefore not be interpreted as an absence of EM diagnostic accuracy or usefulness.

The PICO for this review and the search strategy are provided in an appendix.

Evidence:

Our search for guidelines and reviews for kidney disease resulted in 40 hits after duplicates were removed. In addition, one of the surveyed laboratories sent a reference of a guideline¹⁸ (which was also included in our retrieved references). From these, 36 were excluded on the basis of title and abstract, leaving five potentially relevant studies, for which full texts were reviewed. None were excluded at this stage, so the final inclusion consisted of three guidelines and two non-systematic reviews, which focus on a variety of pathologies.^{18, 74-77}

Figure 19 offers a flow chart with the screening and selection process.

**Figure 19 – Flow Chart for study selection – Kidney Disease**



The articles retrieved focused on different pathologies or groups of pathologies. In the native kidney, three focused on glomerular diseases: two on inflammatory glomerular diseases as a whole (Glomerulonephritis or GN), one on IgM nephropathy, another one on Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits. A further article focused on a condition in the renal allograft called Thrombotic Microangiopathy.

Glomerular diseases make up approximately 20%–25% of the existing cases of renal failure. Nevertheless, among children, adolescents, and young adults, glomerular disease stands out as one of the main factors leading to irreversible kidney damage.⁷¹

The Kdigo (Kidney Disease: Improving Global Outcomes) international/global working group published a guideline in 2021,⁷⁷ which focused on the most common adult and paediatric glomerulonephritis, such as IgAN membranous nephropathy, nephrotic syndrome including minimal change disease and FSGS, and infection-related GN, as well as systemic immunologic diseases (lupus nephritis, ANCA-associated vasculitis, and anti-GBM antibody GN). Very rare GN types, such as fibrillary GN, immunotactoid GN, and IgM GN, for example, were not covered in this guideline, partly because of lacking resources and partly because of the lack of controlled trials to guide treatment. Furthermore, the focus on immune-mediated glomerular disease also meant that other diseases such as amyloidosis and immunoglobulin deposition diseases, Alport syndrome, and thrombotic microangiopathies were excluded from the scope of this guideline.

These guidelines provide recommendations concerning the management of different renal pathologies (which include the strength of recommendation and quality of evidence), but concerning the diagnostic techniques they provide “practice points”. This type of guidance was used when a systematic review was not done or did not yield sufficient evidence to warrant a recommendation. Therefore, practice points are not graded for strength or evidence quality.

The Kdigo Guidelines state, as a practice point, that a kidney biopsy is the “gold standard” for the diagnostic evaluation of glomerular diseases. They also specify that, under some circumstances (illustrated in Figure 20), treatment may proceed without a kidney biopsy confirmation of diagnosis. The authors also point out that proceeding with treatment without morphologic analysis means lacking information obtained from kidney biopsies, such as activity, chronicity, and other unsuspected glomerular, vascular, and/or tubulointerstitial diseases and injuries, which may have prognostic or even therapeutic significance.

They therefore advise that kidney biopsies be performed when the value of the information obtained from the biopsy exceeds the risks entailed.

The Kdigo guideline recommends, through another practice point, that ideally, samples should be studied by light, immunofluorescence, and EM and evaluated by an experienced nephropathologist (see Figure 21). Light microscopy examination should be considered the baseline initial diagnostic evaluation. The working group acknowledge that due to financial constraints and equipment limitations, EM may not be universally accessible. EM is described in the guideline to play a crucial role in delineating the location, extent, and specific characteristics, such as organised substructure of immune or monoclonal deposits, as well as in evaluating foot process effacement, structural alterations in the glomerular basement membrane, and deposition of glycoproteins or lipids. Certain diagnoses, like minimal change disease and immunotactoid deposition disease, rely on EM for accurate identification. In other cases, EM provides valuable descriptive and semi-quantitative information about podocytes and glomerular basement membrane, enhancing diagnostic precision. In settings where EM is unavailable, the Kdigo working group advises to consider establishing relationships to facilitate EM assessments when needed.

The second guideline concerning glomerulonephritic conditions was published in the USA in 2016 by Sethi et al.,¹⁸ and was considered of low quality with respect to its assessment of diagnostic methods. Indeed, it did not explicitly assess and recommend different diagnostic methods, because its aim was to recommend a pathogenesis-based classification and standardise pathology reporting of GN. We nevertheless decided to use this



article as indirect evidence for diagnostic methods. In fact, it is interesting to note that the recommended classification of GN was primarily based on the findings by immunofluorescence (IF), or less commonly IHC, integrated with light microscopy (LM) and EM. The authors consider EM to be a crucial diagnostic tool for glomerular diseases. They cite a study from 1997, which showed that 20% of renal biopsies could not be accurately diagnosed without EM, particularly glomerular diseases.⁷⁸ The guideline recommends processing a sample of renal cortex for EM for all native renal biopsies.

Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits (PGNMID) is a recently described rare pathology among the spectrum of monoclonal gammopathies of renal significance. It is caused by monoclonal immunoglobulin (mlg) deposits in the glomerulus causing kidney injury^{75(Bridoux, 2021 #798, 79} It usually manifests with chronic glomerular disease, altered renal function, and albuminuria.⁷⁹

The clinical manifestations of PGNMID are non-specific: 80% of patients present with haematuria, 60% with oedema, and nearly 50% with nephrotic syndrome.⁸⁰ Two-thirds of patients have renal insufficiency.

PGNMID is common in populations of European descent, with no sex difference, accounting for 0.2%–3.7% of renal biopsies.^{81, 82} The average age of onset is 55 years, but the disease can occur at any age.⁸⁰ Renal prognosis is poor, as the pathophysiology of PGNMID remains unclear and its treatment challenging.⁷⁹ However, the use of biological agents targeting clones have greatly improved the prognosis of PGNMID patients.^{75, 79}

Early diagnosis of PGNMID is crucial for a better prognosis, and, according to the non-systematic review by Lin et al., diagnosis is based on comprehensive clinical evaluations, complete renal biopsies, and in-depth immunological and haematological examinations. More thorough investigations may also be needed with a series of immunological and haematological examinations (e.g., bone marrow biopsy, PET-scanning) required to determine whether there is pathological plasma cell or lymphocyte disease. The review describes the pathological diagnostic criteria for PGNMID in terms of light microscopy, immunofluorescence, and

EM, without specifying whether these three techniques should always be used or whether there are situations in which not all of them are necessary.

The third condition in the native kidney which we retrieved a (non-systematic) review on, is IgM Nephropathy (IgMN).⁷⁶ IgM Nephropathy is a controversial clinicopathological entity characterized by IgM deposits in the mesangium at immunofluorescence with a very varied histological picture. There is no consensus about the significance of the presence of IgM deposits, and the interpretation of IF findings is controversial.⁸³ Clinically, it presents as nephrotic syndrome, asymptomatic proteinuria and/or isolated haematuria. These characteristics narrowly define IgMN between minimal change disease and focal segmental glomerulosclerosis, so it is not often considered as a separate pathology. Therefore, homogeneous epidemiologic and pathologic data are not available.⁸³ The frequency of IgMN in native renal biopsies varies from 2 to 18.5 %.^{76, 83-85} The disease is more common in children than in adults.

According to Mubarak et al.'s 2011 review, the diagnosis of IgMN depends on detailed pathologic examination of kidney biopsy by LM, immunofluorescence, and EM. Of these, the first two are considered essential, while EM is optional. The authors also state that the pathologic findings must in addition be put into perspective with clinical and serological investigations to exclude secondary causes of IgM deposition.⁷⁶

The fourth condition captured by our searches was Thrombotic Microangiopathy in the Renal Allograft.⁷⁴ This disease is characterised by endothelial injury, marked by the thrombotic occlusion of small vessels, leading to transplant failure that is frequently clinically unforeseen.⁷⁴ Various factors, including immunologic, genetic, hematologic disorders, and drugs, can potentially trigger this disorder.^{74, 86, 87} A conclusive diagnosis is obtained by kidney biopsy⁸⁸ but accurate diagnosis is often complicated by the diverse range of morphologies, often causing delays in the initiation of targeted therapy.



The 2023 consensus guideline by the international Banff TMA working group (Afrouzian et al. 2023)⁷⁴ concerns the development of minimum diagnostic criteria of Thrombotic Microangiopathy in the transplanted kidney, and reports on the first phase (the pathology phase) of the Delphi method.⁷⁴ The consensus on diagnostic criteria stem from a combination of exams, including clinical, laboratory, and pathology (light microscopy, EM, and immunofluorescence). The authors do not provide any further insights into the comparative accuracy of the different pathological techniques, and whether application of all techniques is warranted in every case.

It therefore serves us as merely as indirect evidence about the use of EM in the diagnosis of TMA. Because the focus of the consensus did not exactly coincide with our research question, and because it was not a clinical guideline per se, but rather the report on a first Delphi round, we were not able to assess the quality of the article through the AGREE tool. A more complete diagnostic flow may possibly be provided in the final guideline after completion of all Delphi phases (the second phase with nephrologists is already underway but the results have not been published yet).

Table 33 – Guidelines and Reviews on Kidney Disease

Title	Thrombotic Microangiopathy in the Renal Allograft: Results of the TMA Banff Working Group Consensus on Pathologic Diagnostic Criteria	A Review on the Diagnosis and Treatment of Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits.	IgM nephropathy revisited.	Mayo Clinic/Renal Pathology Society Consensus Report on Pathologic Classification, Diagnosis, and Reporting of GN.	KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases
Authors	Afrouzian M et al.	Lin L et al.	Mubarak M & Kazi JI	Sethi S et al.	Kidney Disease: Improving Global Outcomes (KDIGO)
Publication year	2023	2022	2011	2016	2021
Disease group	Thrombotic Microangiopathy in the Renal Allograft	Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits	IgM nephropathy	Glomerulonephritis	Glomerular Disease
Study type	Consensus Guideline	Non-systematic review	Non-systematic review	Consensus Guideline	Clinical Practice Guideline
Country*	International	China	Pakistan	USA	International



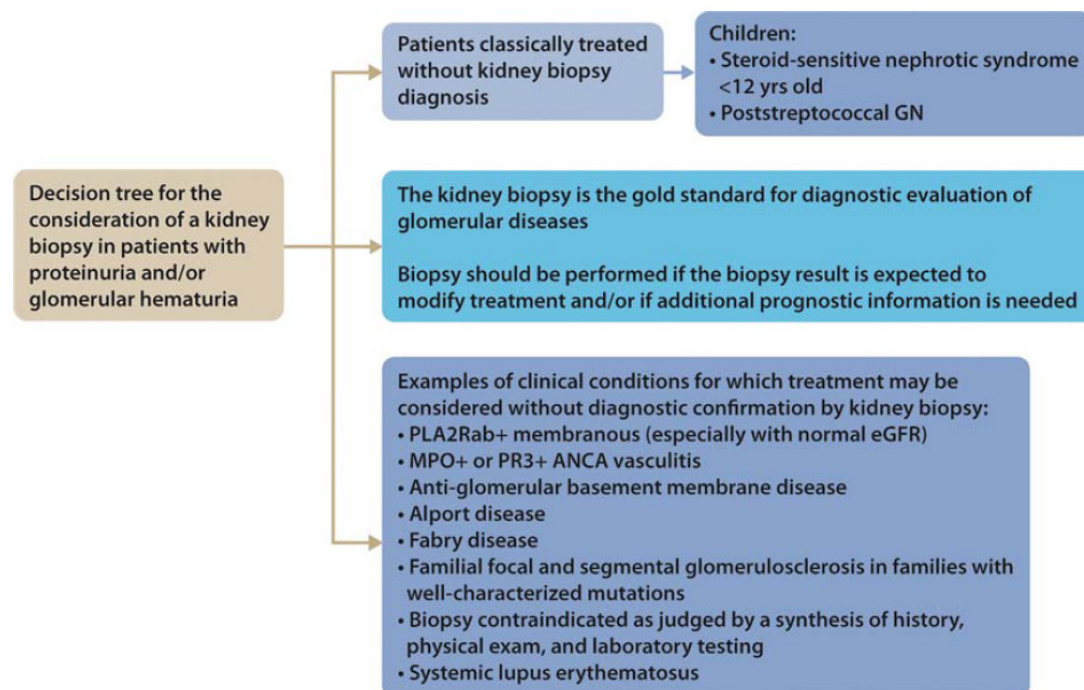
Methods	Development of minimum diagnostic criteria of Thrombotic Microangiopathy in the renal allograft using Delphi method for consensus generation, including histological evaluation of real-life cases. This article reports only on Phase I of the study (pathology phase)	Unclear. No methodology section.	Very few details on methodology. PubMed search.	A group of renal pathologists and nephrologists met at the Mayo Clinic on February 20, 2015 to reach a consensus on the standardised classification and reporting of GN (N.B. main question was not to assess diagnostic tools, neither to provide a workflow for diagnosis. The use of IF, LM, and EM as diagnostic tools appears as an assumption in this article). The focal point of the meeting was to recommend an aetiology/pathogenesis-based classification of GN and standardise pathology reporting of GN.	Update of the KDIGO 2012 guideline. Systematic reviews and use of GRADE for quality assessment. Use of AGREE II reporting checklist. Commission composed of paediatric and adult nephrologists, pathologists, epidemiologists, and methodologists. Cochrane subgroup contracted to carry out systematic reviews. Focus mainly on treatment rather than assessment of best diagnostic technique.
Diagnostic tests	A combination of exams: clinical, laboratory (venous blood tests), and pathology (light microscopy, EM, and immunofluorescence)	Diagnosis is based on comprehensive clinical evaluations, complete renal biopsies, and in-depth immunological and haematological examinations. Pathological diagnostic criteria for PGNMID are described in terms of light microscopy, immunofluorescence, and EM.	The diagnosis of IgMN depends on detailed pathologic evaluation of renal biopsy by LM, immunofluorescence, and EM. Of these, the first two are essential, and EM is optional. It confirms the LM and IF findings. The pathologic findings need to be correlated with clinical and serological studies to exclude the secondary causes of IgM deposition	The classification of GN is primarily recommended on the basis of the findings by IF (or less commonly IHC), integrated with LM and EM. EM is considered a crucial diagnostic tool for glomerular diseases. Processing a sample of renal cortex for EM is recommended for all native renal biopsies.	Serology and urinalysis, kidney biopsy as gold standard for diagnosis. Ideally, all kidney biopsies should be assessed by light microscopy, immune-histology, and EM.
EM part of diagnostic pathway?	Yes, no precise order or prioritisation of investigations was specified.	Diagnosis relies on renal biopsy. The article doesn't clarify how the biopsy should be analysed.	According to authors, EM evaluation of biopsies is optional.	Yes, no precise order or prioritisation of investigations was specified.	Yes



Strength of recommendation	Not reported	Not reported	Not reported	Not reported	Low (Clinical practice point)
Quality of evidence	Not reported	Not reported	Not reported	Not reported	Not reported
Quality of the study	Could not be assessed	Could not be assessed	Could not be assessed	Low (for diagnostic methods)	High

*For reviews this corresponds to the author affiliation

Figure 20 – Kdigo Practice Point: Decision tree for the consideration of a kidney biopsy

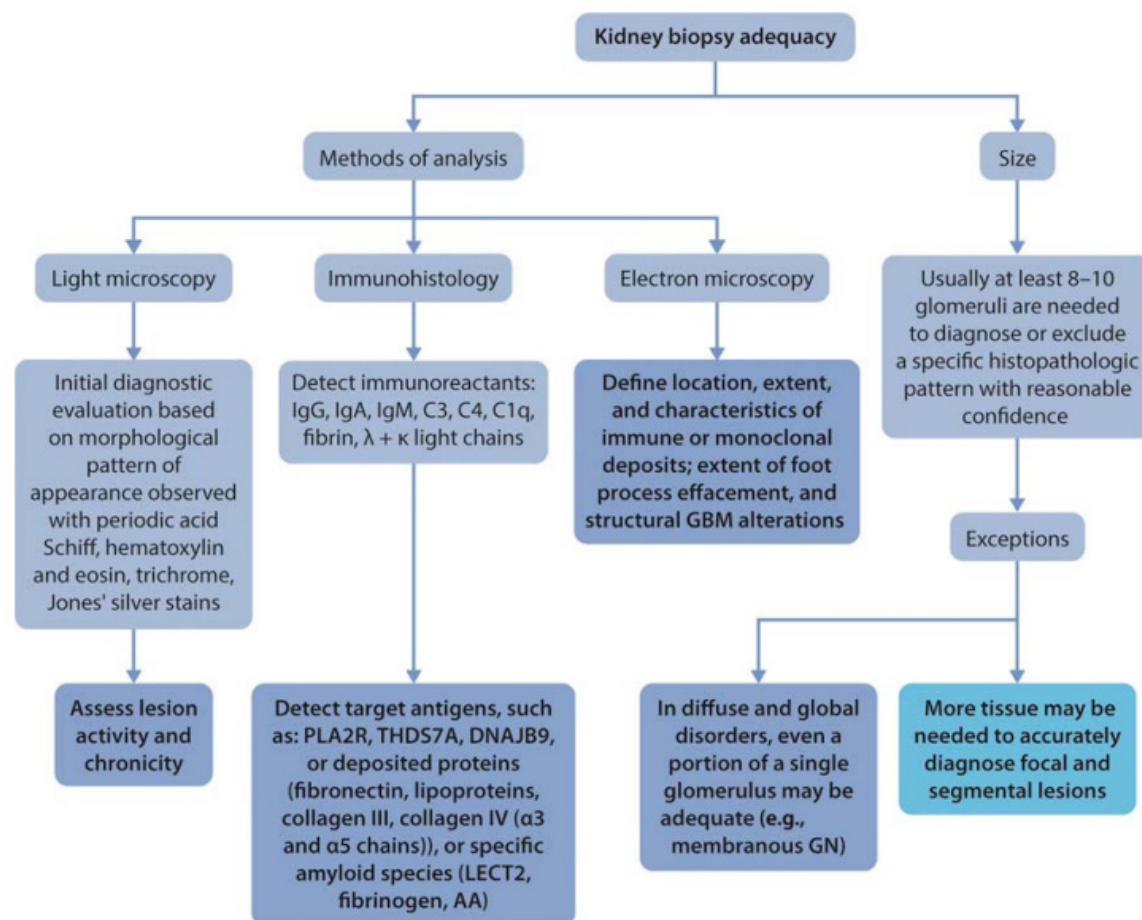


Considerations for a kidney biopsy in patients with proteinuria and/or glomerular hematuria. ANCA, antineutrophil cytoplasmic antibody; eGFR, estimated glomerular filtration rate; GN, glomerulonephritis; MPO, myeloperoxidase; PLA2Rabp, M-type phospholipase A2 receptor antibody positive; PR3, proteinase 3.

Source: Kdigo 2021 Clinical Practice Guideline for the Management of Glomerular Diseases⁷¹



Figure 21 – Kdigo Practice Point: The evaluation of kidney tissue should meet standards of biopsy adequacy



Evaluation of kidney tissue. AA, amyloid A; GBM, glomerular basement membrane; DNAJB9, DnaJ homolog subfamily B member 9; GN, glomerulonephritis; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LECT2, leukocyte cell-derived chemotaxin-2; PLA2R, M-type phospholipase A2 receptor; THDS7A, thrombospondin type-I domain-containing 7A.

Source: Kdigo 2021 Clinical Practice Guideline for the Management of Glomerular Diseases⁷⁷



3.4.2 *Electron microscopy in the diagnosis of Ehlers Danlos Syndrome*

Ehlers-Danlos syndrome (EDS) are a group of inherited connective tissue disorders which manifest with varying degrees of severity and varying features.⁸⁹ These can include skin hyperextensibility, joint hypermobility, easy bruising, fragility of skin, and, in some cases, rupture of soft tissues, blood vessels or internal organs. Significant discoveries in the genetic basis of EDS have led to a revised classification into thirteen (and more recently fourteen) disease subtypes in 2017 by the International EDS Consortium.⁹⁰ ⁹¹ For thirteen of the fourteen subtypes the molecular cause is known. These types are caused by variants in 20 different genes. However, the molecular underpinnings of the hypermobile type of EDS remain unknown.

The existence of numerous subtypes highlights the vast genetic and clinical variability, which, until the recent advances in genetic testing, complicated the diagnostic process, leading to clinical uncertainty and diagnostic confusion.

The prevalence of Ehlers-Danlos syndrome is estimated to be between 1 in 10 000 and 1 in 100 000, depending on the EDS subtype, although this could be an underestimation.⁹² The accurate prevalence of the different EDS subtypes is still not known because most data predates the new 2017 classification. What there is consensus on is that hypermobility type EDS is the most common subtype, with an incidence between 1 in 10 000 and 1 in 15 000.^{93, 94}

The management of EDS involves the monitoring of significant complications, both major and organ-specific (such as arterial aneurysm and dissection), along with physical therapy and rehabilitation. Currently, there are no particular medical or genetic therapies accessible for any EDS type.⁹¹

Evidence

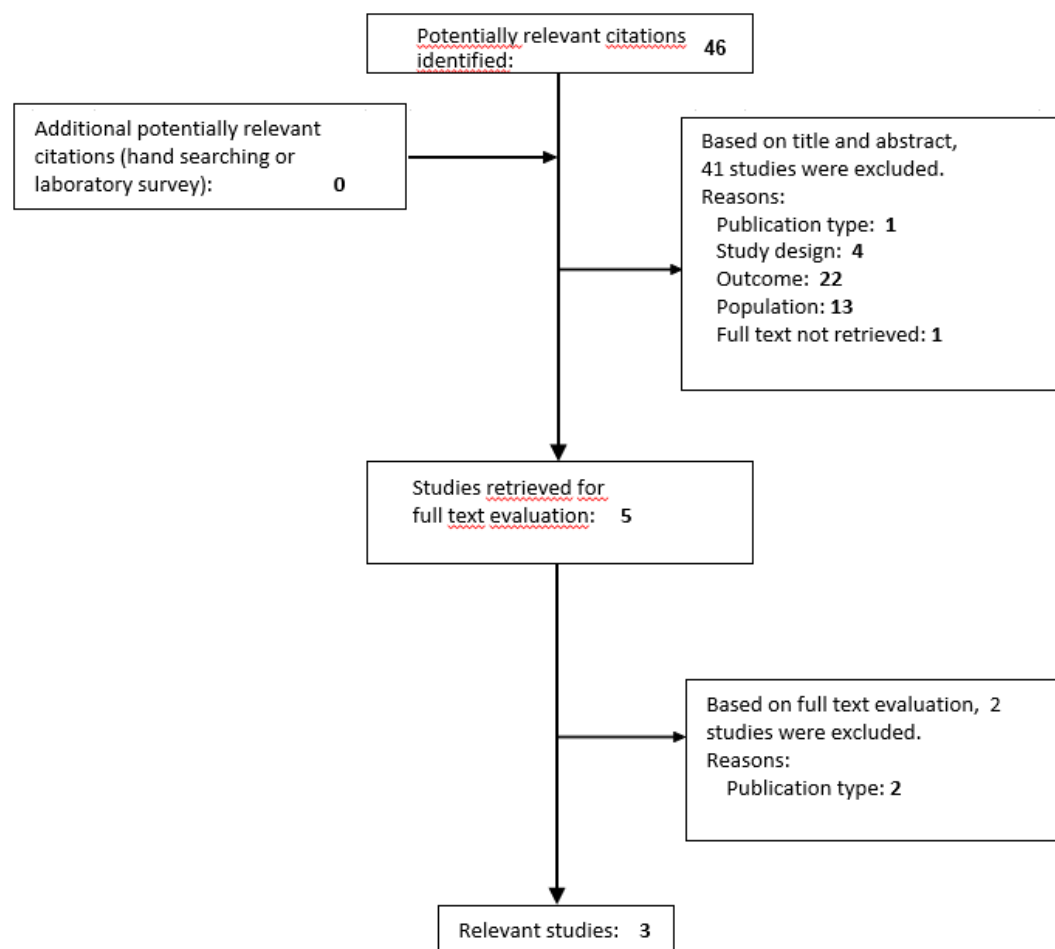
The PICO for this review and the search strategy are provided in appendix.

Our search for guidelines and systematic reviews resulted in 46 hits after duplicates were removed. From these, 41 were excluded on the basis of title and abstract, leaving five potentially relevant articles, for which full texts were retrieved for consideration. After full text review, two were excluded because they were published comments rather than guidelines or systematic reviews.

The three retained articles were one systematic review and two articles with no specified methodology. One of these was the 2017 International EDS Consortium revised classification of EDS types and the second was a general review. Figure 22 offers a flow chart with the screening and selection process. Due to lacking methodological information, the quality of two articles (Malfait 2017, Sobey 2015) could not be assessed. The systematic review by Doolan BJ et al. was assessed using the AMSTAR 2 online tool⁹⁵ and the assessment is reported in the appendix.



Figure 22– Flow Chart for study selection – Ehlers Danlos syndrome





While the earliest of the three retained studies, a non-systematic review by Sobey et al. published in 2015⁹⁶, still recommended skin biopsy together with electron microscopy analysis as first line examination for certain subtypes (e.g. classical EDS), the two more recent articles (one British systematic review published in 2023⁹⁷, and the 2017 international classification of EDS⁹⁰) considered genetic testing (e.g. next-generation sequencing) the gold standard diagnostic tool for all EDS subtypes, except hypermobile EDS which is considered a purely clinical diagnosis. Nevertheless, both articles consider transmission electron microscopy (TEM) to be useful to support diagnosis when genetic results are unclear or inconclusive. Indeed, there are instances where genetic analysis might not provide a definitive outcome or detect variants with uncertain significance. In such situations, supplementary evidence may be necessary to establish a more convincing diagnosis of EDS. According to Doolan et al., TEM is useful in supporting the case for variants classified as uncertain significance.⁹⁸

However, the authors also point out that TEM may yield false negative results, that findings may not be correlated to the clinical severity, and that they mostly do not allow to identify the specific EDS subtype.^{97, 99, 100} Furthermore, since abnormalities observed through TEM are generally not exclusive to Ehlers-Danlos Syndrome, they do not serve as conclusive confirmation for the diagnosis.⁹⁰ Furthermore, TEM may also support the clinical diagnosis in instances where genetic testing is not accessible. Yet, according to Malfait et al., obtaining a molecular diagnosis holds paramount importance for counselling purposes, as it enables the validation of the specific diagnosis. It provides insights into the inheritance pattern, recurrence risk, prognosis, and can offer guidance for management.⁹⁰

Additionally, for certain specific EDS subtypes (e.g. kyphoscoliotic and spondylodysplastic EDS) analysis of urine deoxypyridinoline/pyridinoline crosslink ratios was considered part of the diagnostic pathway.^{90, 97}


Table 34 – Guidelines and Reviews on Ehlers Danlos

Title	Dermatologic manifestations and diagnostic assessments of the Ehlers-Danlos syndromes: A clinical review	The 2017 International Classification of the Ehlers–Danlos Syndromes	Ehlers–Danlos syndrome: how to diagnose and when to perform genetic tests
Authors	Doolan BJ et al.	Malfait F et al.	Sobey G
Publication year	2023	2017	2015
Disease group	EDS	EDS	EDS
Study type	Systematic Review	Unclear	Non-systematic review
Country*	UK	International	UK
Methods	Systematic review for each different EDS subtype. PubMed & Web of Science . Search: March 2017- 15th January 2022	The study proposes a new (clinically-oriented) classification of EDS into 13 subtypes. No methodological details.	No methods specified
Diagnostic tests	DNA analysis (next-generation sequencing) is the gold standard. If GNS results are not clear or conclusive, additional investigations such as TEM can help make a diagnosis. Urine analysis is useful for diagnosis of specific EDS subtypes.	DNA analysis for all subtypes, except for hypermobile (hEDS). hEDS diagnosis rests on clinical findings. For certain subtypes, molecular screening by means of targeted resequencing of a specific gene panel is indicated. For various subtypes, in case of unavailability of genetic testing, specific TEM findings on skin biopsy can support the clinical diagnosis but cannot confirm it.	Skin biopsy analysed via histology, EM, or fibroblast culture, is helpful in patients where particular subtypes are considered. Classical EDS suspicion: skin biopsy for EM is the recommended first investigation; if positive findings next step is molecular genetic testing. For other EDS subtypes, diagnostic tools include genetic testing, urine analysis, and TEM.
EM part of diagnostic pathway?	Yes in few cases to assist diagnosis	Yes in few cases to assist diagnosis	Yes, depending on subtype
Strength of recommendation	Not reported	Not reported	Not reported
Quality of evidence	Not reported	Not reported	Not reported
Quality of the study	Low quality	Could not be assessed	Could not be assessed



3.4.3 *Electron microscopy in the diagnosis of Primary Ciliary Dyskinesia*

Primary Ciliary Dyskinesia (PCD) is a rare genetic condition affecting cilia structure and causing dysfunctional motility which impairs mucociliary clearance. Diverse mutations give rise to distinct clinical and pathological patterns, which complicates diagnosis.

The clinical presentations include respiratory distress and situs invertus in newborns, chronic wet cough, recurrent respiratory tract infections, or bronchiectasis in children, and infertility in adults. Patients mostly experience long-term rhinorrhoea, chronic wet cough, progressive loss of lung function and eventually develop structural damage of the airways, and bronchiectasis.^{101, 102} The prevalence of diagnosed PCD in European countries has been estimated at 3-7 per 100 000 in children and 0.2-6 per 100 000 in adults.¹⁰³

We reviewed the literature for existing evidence on diagnostic tools for PCD. The PICO and search strategy for this review are provided in the appendix.

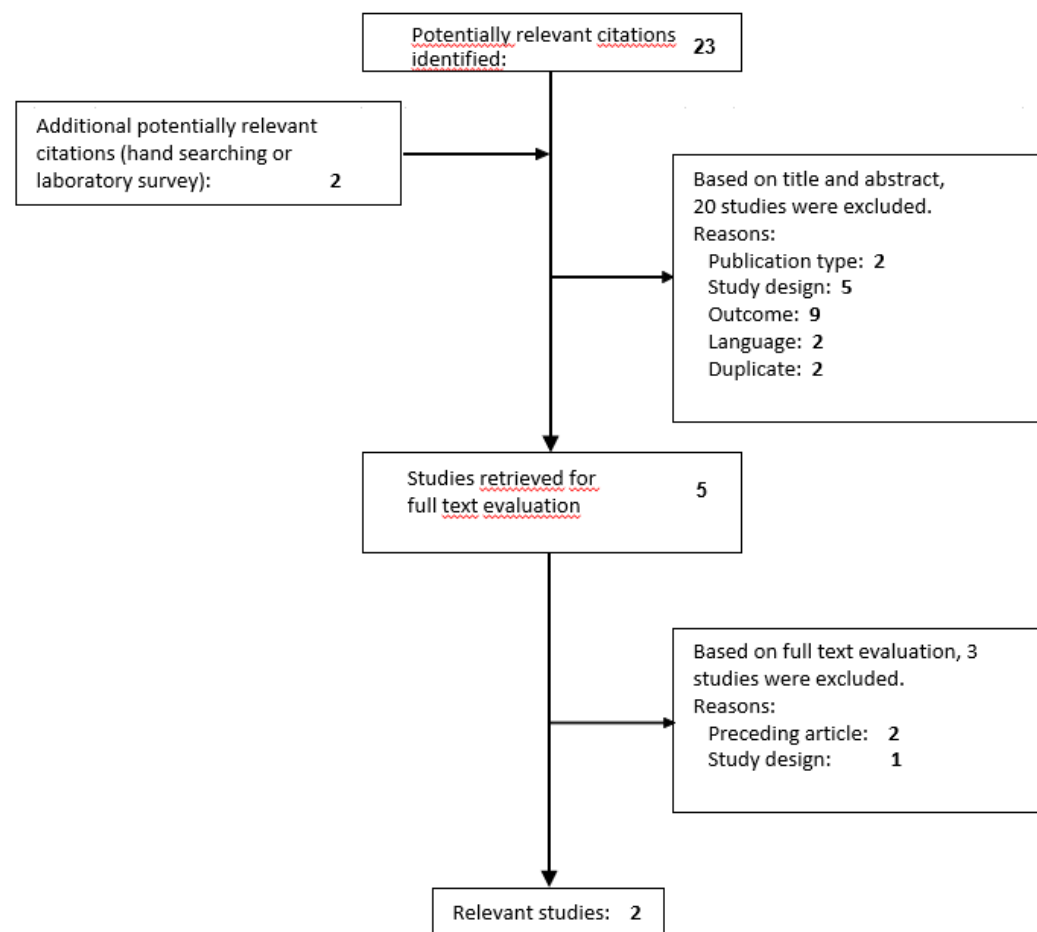
Evidence

Our search for guidelines and systematic reviews for Primary Ciliary Dyskinesia resulted in 23 hits after duplicates were removed, and 2 additional references were provided by the surveyed laboratories. These two references^{20, 104} had already been included in the retrieved references, so they were removed as duplicates. From the remaining 23, another 18 were excluded on the basis of title and abstract, leaving 5 potentially relevant guidelines, for which full texts were retrieved for consideration. Two were excluded because they were earlier guidelines or consensus recommendations that were updated by the guidelines we retained. One article was excluded because it was a guideline review.

The 2 retained articles are clinical practice guidelines, one by the European Thoracic Society published in 2017¹⁰⁴, and the other by the American Thoracic Society published in 2018¹⁰⁵. Figure 23 offers a flow chart with the screening and selection process.



Figure 23 – Flow Chart for study selection – Primary Ciliary Diskinesia





According to Shapiro et al., for more than forty years, the diagnosis of PCD relied on identifying structural defects in the ciliary axoneme through Transmission EM analysis.¹⁰⁵ However, they explain that this method has significant drawbacks. Nonspecific ciliary changes, which can result from other causes (e.g. exposure to environmental pollutants or infection), may resemble the abnormalities observed in PCD. Additionally, the absence of axonemal defects does not rule out PCD, as 30% of affected individuals exhibit normal ciliary ultrastructure.^{101, 105} More recently, newer diagnostic methods have been developed, which include nasal nitric oxide (nNO) measurement, genetic testing, digital high-speed video microscopy with ciliary beat pattern analysis (HSVA), and immunofluorescence imaging.¹⁰⁵ Both guidelines included in our review were considered of high quality (using the AGREE GRS tool). They discuss the different diagnostic methods and propose a diagnostic algorithm (Figure 24). Both guidelines highlight that there is no “gold standard” PCD diagnostic test and that diagnosis relies on a combination of (technically demanding) diagnostic tools.

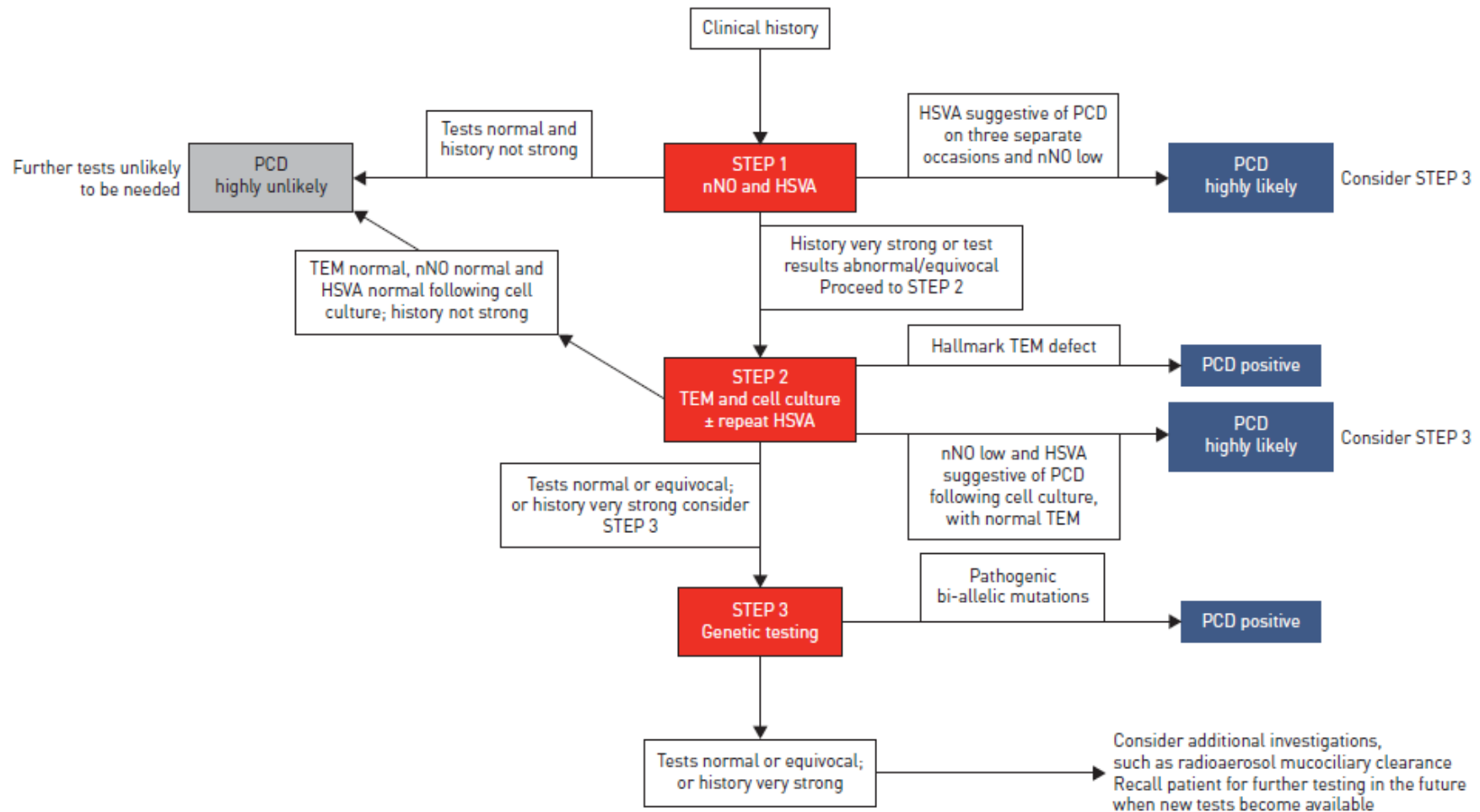
The two guidelines suggest a slightly different algorithm, both recommending nasal nitric oxide measurement in first instance, and TEM as a second line test, to be used in specific cases. Nevertheless, the criteria for the use of TEM differ, as well as the recommendations for the situations in which to use genetic testing and HSVA.


Table 35 – Guidelines and Reviews on Primary Ciliary Dyskinesia

Title	ERS Task Force guideline for the diagnosis of primary ciliary dyskinesia	Diagnosis of Primary Ciliary Dyskinesia. An Official American Thoracic Society Clinical Practice Guideline
Authors	Lucas JS et al.	Shapiro et al.
Publication year	2017	2018
Disease group	PCD	PCD
Study type	Clinical Practice Guideline	Clinical Practice Guideline
Country	Europe	USA
Methods	Systematic review of the literature, and assessment of the evidence using GRADE. Modified Delphi survey to develop an algorithm of diagnostic tests.	Systematic reviews + meta-analyses when pertinent to summarise evidence. Evidence assessed through GRADE & discussed with multi-disciplinary panel.
Diagnostic tests	Decision to test for PCD is taken based on specific clinical criteria. If these are met, nasal nitric oxide measurement and High Speed Video Analysis (HSVA) are first step investigations. Some patients should proceed to step 2 (TEM). Step 3: Genetics testing and or repeat HSVA (with or without cell culture)	In patients presenting at least 2 of the 4 key clinical features for PCD, further testing includes: if available and patient cooperative (and at least 5 years old) nNO, otherwise extended genetic testing panel. TEM or ciliary ultrastructure are used second line in specific cases (see algorithm)
EM part of diagnostic pathway?	Yes	Yes
Strength of recommendation	Strong recommendation	Not reported
Quality of evidence	Based on low confidence in evidence	Not reported
Quality of the study	High	High



Figure 24 – European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia.



Source: Lucas et al. 2017 ¹⁰⁴



3.4.4 *Electron Microscopy in muscle diseases*

Muscle diseases or myopathies, are a heterogeneous group of disorders which encompass both hereditary genetic and acquired disorders, occurring in both childhood and adulthood. They commonly cause muscle weakness, stiffness, pain, or spasms. Myopathies can be classified into various types based on their causes, including inflammatory, congenital, mitochondrial, or metabolic myopathies for instance.¹⁰⁶

While the previously analysed NIHD report data, together with the laboratory survey, revealed the widespread use of EM in the diagnosis of (rare) myopathies (e.g. metabolic myopathies, neuromuscular disorders), our literature searches for diagnostic guidelines and systematic reviews (see appendix for details) retrieved only two references, which were excluded because of wrong study design (case-study) and wrong focus. The latter article provided a description of the ultrastructural defects in mitochondrial myopathies, highlighting the link between mitochondrial ultrastructure and function.¹⁰⁷ The article did not aim to provide an assessment of the accuracy of EM in clinical diagnostics of mitochondrial diseases (e.g. through a comparison with other diagnostic tools), but nevertheless confirms the technique's ability of capturing ultrastructural defects. In our laboratory survey, one respondent had provided a reference¹⁹ in the context of neuromuscular diseases; however, this was a guideline on nerve biopsies, which are rarely carried out (as described in Section 2.3.2) and which were therefore not covered by our literature review. The respondent also suggested the European Reference Network for rare or low prevalence complex diseases as a resource for potential guidelines. We searched this website and found numerous (17) guidelines on neuromuscular diseases. However, as explained in the methods section (Section 3.1.2), we could not assess individual guidelines for every single rare disease and did not identify any general guideline on the use of EM or other diagnostic techniques in (rare) muscular diseases more generally.

We further contacted pathologists specialised in EM, and received a response by one pathologist expert in muscle biopsy analysis, who provided some background information, together with three articles on muscular diseases, which we will describe below.

The expert also pointed out that the use of muscle biopsies in the diagnosis of neuromuscular disorders has changed considerably over the last decade. This is mainly due to the advent of genetic techniques such as whole exome sequencing and whole genome sequencing, that can be used to diagnose many congenital and familial muscle disorders, while earlier their diagnosis used to rely more on pathology. Nevertheless, he believed that several indications remain for muscle biopsy, even after thorough genetic investigations, for instance to prove the pathogenicity of some genetic alterations.

Below, we describe (and summarise in Table 32) the relevant information contained in the three articles that we retrieved, namely one German guideline on the diagnosis of myopathies¹⁰⁸, one consensus recommendation on the standards for muscle pathology¹⁰⁹, and one non-systematic review on muscular diseases in neuropathology¹¹⁰.

The guideline by Deschauer et al. (low quality) published in 2021 for the Guidelines Commission of the German Society for Neurology provides an overview of existing diagnostic methods, which, in combination, guide the diagnosis of different muscular pathologies. The guideline unfortunately does not provide a diagnostic algorithm, nor does it provide specific and graded recommendations to specific research questions.

The authors describe the baseline diagnostic investigations, including laboratory tests, neurophysiological tests, and imaging, and state that molecular genetic testing in cases of suspected myopathy has become an essential component of the diagnostic process, often employed relatively early, making it increasingly possible to forego invasive muscle biopsies. The identification of the gene defect is also becoming more crucial from a therapeutic perspective, as an increasing number of gene-specific therapeutic approaches emerge, as seen in conditions such as Duchenne muscular dystrophy. The rapid advancements in human genetics, particularly through novel methods like next-generation sequencing (NGS), have revolutionised the diagnosis of genetically related disorders. However, these methods also have their limitations and must be judiciously applied. If no gene defect can be identified, a muscle biopsy is indicated.



Even with the detection of sequence variants of unclear significance, a muscle biopsy may be necessary to establish pathogenicity.

In metabolic and mitochondrial muscle diseases, the guideline considers that biopsy continues to play a central role in diagnosis. In cases of suspected myositis, muscle biopsy remains the gold standard for confirming the diagnosis.

The review emphasises that muscle biopsy specimens must be available for histological examination, but potentially also immunohistochemistry, Western blot, enzymatic measurements, electron microscopy, and DNA analyses. Electron microscopic examinations are considered helpful for detecting for instance tubulofilamentous inclusions in inclusion body myositis and for analysing characteristic structural changes in congenital myopathies and diagnosing sporadic late-onset nemalin myopathy.¹¹¹ The guideline recommends that muscle samples be examined at a specialised centre.

The (non-systematic) literature review published by Schänzer et al. in 2022¹¹⁰ describes the ultrastructural features in various muscular diseases of both childhood and adulthood, which can aid in further delineating certain muscle diseases. The review therefore does not assess or compare different diagnostic methods, and instead considers the investigation by EM of a muscle biopsy as the starting point for the description of the ultrastructural findings.

The authors nevertheless state that the value of EM in the diagnosis of neuromuscular disorders is provided by the fact that clinical and genetic findings are not always conclusive or clear-cut. They consider muscle pathology to be helpful in these situations to elucidate the underlying cause of the disease or provide support for a molecular panel diagnosis. Additionally, the findings can facilitate the assessment of whether a new gene variant should be classified as pathogenic. They consider that in addition to processing muscle biopsies with specific enzymatic and immunohistochemical techniques, fixed tissue for electron microscopic analysis should always be provided in a muscle biopsy as EM evaluation

can be helpful in supporting a diagnosis and better delineating the pathogenesis.

Despite automation, the authors recognize that preparation of samples for transmission electron microscopy remains very time-consuming, and that the assessment requires high expertise and is therefore not conducted in all neuropathological institutes.

The third article on the subject of muscular disease diagnosis is a 2019 publication by the EURO-NMD pathology working group, which recommends Standards for Muscle Pathology.¹⁰⁹ EURO-NMD is the dedicated European Reference Network (ERN) for neuromuscular patients, which was set up to improve the access to accurate diagnostics and disease management for patients with rare disorders. All the hundreds of different neuromuscular disorders are indeed rare diseases. A 2017 survey by this group revealed a considerable variability in the methods employed by pathology laboratories, underscoring the need for recommended standards in muscle pathology procedures and analyses. This publication unfortunately did not include sufficient methodological information for us to assess its quality. Similarly to the article described above, the focus of the study was not to assess or compare different diagnostic methods, as the pathological investigation of muscle biopsies was considered the starting point. The authors recommend that EM be used in samples without diagnostic conclusions through other methods, to clarify abnormalities observed or not visible on light microscopy. They consider that this applies particularly to suspected congenital myopathies, unclear sarcoplasmic abnormalities, dysimmune, toxic, mitochondrial, metabolic myopathies, in neonatal muscle biopsies, and optionally in neuromuscular junction in myasthenic syndromes.

They consider that when processing the biopsy, samples should always be available in order to be used, when necessary, for essential techniques including histology, enzyme histochemistry, immunohistochemistry, Western blotting, and electron microscopy.

**Table 36 – Guidelines and Reviews on myopathies**

Title	Leitlinien für Diagnostik und Therapie in der Neurologie. Diagnostik von Myopathien	Neuropathologie I: Muskuläre Erkrankungen	1st ENMC European meeting: The EURO-NMD pathology working group Recommended Standards for Muscle Pathology
Authors	Deschauer M et al.	Schänzer A et al.	Udd B et al.
Publication year	2021	2022	2019
Disease group	Myopathies	Muscular diseases	Muscle pathology
Study type	Guideline	Review	Consensus recommendations
Country*	Germany, Austria, Switzerland	Germany	International
Methods	Systematic review in PubMed (no search strings provided) and virtual conference with expert neurologists and representatives of German, Austrian, and Swiss neurology and genetic professional societies.	The study proposes. No methodological details.	Based on a 2018 preliminary draft reviewed by 32 muscle pathology experts, and further discussed in a ENMC sponsored meeting on 7 December 2018.
Diagnostic tests	Laboratory tests, neurophysiological tests, imaging, molecular genetic testing, muscle biopsy (analysed through histology, and potentially also immunohistology, Western blot, enzymatic measurements, electron microscopy, and DNA analyses)	Genetic testing, processing muscle biopsies with specific enzymatic and immunohistochemical techniques. In addition, electron microscopy can be helpful in supporting a diagnosis.	Histology, enzyme histochemistry, immunohistochemistry, Western blotting, and electron microscopy
EM part of diagnostic pathway?	Yes, when muscle biopsy is indicated because diagnosis is not conclusive based on laboratory, imaging, neurophysiologic tests, and possibly genetic tests alone. Depending on the suspected type of myopathy, EM can be a useful pathologic technique to provide a diagnosis.	Yes, in cases where clinical and genetic findings are not conclusive or clear-cut. The authors state that tissue for EM should always be provided in a muscle biopsy, but it is not clear whether they consider that it should always be analysed with EM or only when other pathology techniques do not yield conclusive results.	Yes, in samples without diagnostic conclusions through other methods. Particularly in suspected congenital myopathies, unclear sarcoplasmic abnormalities, dysimmune, toxic, mitochondrial, metabolic myopathies, in neonatal muscle biopsies, and optionally in neuromuscular junction in myasthenic syndromes.
Strength of recommendation	Not reported	Not reported	Not reported
Quality of evidence	Not reported	Not reported	Not reported
Quality of the study	Low quality	Could not be assessed	Could not be assessed



Conclusion – Guidelines and reviews on EM diagnostic indications

- Diagnostic EM use is commonly recommended in a limited number of indications: kidney disease (e.g. glomerulopathies), muscular diseases, and Primary Ciliary Diskinesia (PCD).
- Except for PCD, few (high-quality) guidelines or systematic reviews were identified in our searches.
- EM is recommended in the PCD diagnostic pathway and for the diagnosis of specific renal diseases (e.g. glomerulonephritis). In muscle pathology high-quality studies are lacking, but existing evidence supports the use of EM, particularly when genetic testing is inconclusive or other pathology techniques (e.g. LM) cannot provide an answer. In addition, EM is (only) recommended as a supportive tool for suspected EDS when first-line diagnostic methods did not yield clear results. When hypermobile EDS is suspected, this should be clinically diagnosed.
- EM should be carried out in specialised centres.

4 CONCLUSIONS AND DISCUSSION

Our research presents a first attempt to describe the use of IHC and EM testing in Belgium, in order to identify the indications where such use is more prominent, as well as trends over the last decade, highlighting possible causes of growth and offering recommendations on how to ensure increased transparency in their use for future evaluations and analyses.

This work was carried out at the request of the NIHDl, following a significant increase of expenditure in the area, over the last decade.

Our work focuses on EM use, as well as on diagnostic IHC staining.

Diagnostic IHC appears to account for the large majority of IHC use (95% in 2019) and can be used both for oncological and non-oncological diagnoses. Given the impossibility of studying all existing medical areas of IHC use, and the more extensive availability of guidance for IHC use in the oncology field, case studies were performed for two non-oncologic indications: i.e. detection of *H. pylori* and diagnosis of coeliac disease. The selection of these areas was performed following the identification of GI as the most frequent medical specialty for IHC use. It is important to highlight that any insights gained via these case studies into factors influencing the use of IHC, as well as general data gaps, may also be informative for other indications.

General use of IHC in Belgium

According to our analysis, the total public (NIHDl) expenditure associated with IHC stains grew 103.6% between 2012 and 2021. This represents four times the growth of the total expenditure in pathology over the same period (25.7%).



Overall, IHC use also grew over the last 10 years: from 752.900 stains in 2012 to 1 278 460 stains in 2019, which correspond to an increase of 69.8%. Only in the 2020 (covid pandemic year), a small reduction in IHC staining was perceived. The growth is apparent in all different indications/medical areas explored (GI biopsies, skin biopsies, gynaecological biopsies, skin biopsies, breast sampling, prostate biopsies, surgical procedures with biopsies). Both the number of procedures in which at least one IHC stain has been performed, as well as the mean number of stains performed per sampling have increased.

During discussion with experts in the field, a point was raised regarding the fact that the population has increased in the past decade and more importantly, the age composition of the population is slowly changing, with more people over 18 (adults), and over 65 (elderly). The extent to which these factors may have influenced the growth in IHC was thought to be an interesting point to investigate. Thus, a correction of our data analysis on that basis was attempted, but resulted in a similar increase in IHC use (+66.7). Such growth does not appear to be associated to an increase in the number of procedures, or to the number of biopsies studied over that same period. This result was discussed during an expert meeting to highlight possible explanations for the relative differences in the evolution of procedures and biopsies versus that of IHC use. A limitation in the current nomenclature coding linked to biopsies was highlighted. Data currently registered on biopsies do not discriminate according to the number of individual tissue samples taken, whether one or more, since this will simply be captured as one in the Belgian administrative data. Thus, the extent to which the growth in IHC staining may respond to an increase in the number of individual tissue samples taken, remains unclear. A check was done on the recommendations regarding the number of biopsies reflected in the clinical guidelines included in our review of the literature, to see if such recommendations had evolved over the last decade. For coeliac disease, recommendations appear to remain similar in guidelines recently published^{53, 64} than in recommendations published in 2008.¹¹² Similarly, for *H. pylori* the number of recommended biopsies seems to remain stable, with the updated Sydney system recommending 5 biopsies in 1994¹¹³, similarly to the most recent European guidelines.⁹ However, given the focus of our literature reviews being limited to *H. pylori* and coeliac disease, as well as

the possibility that standard practice may have evolved, independently of the existing guidelines, this check remains purely informative but inconclusive.

A crucial limitation of our data analysis, is given by the fact that no indication for IHC tests is currently available in the existing Belgian administrative databases. An approximation to the most frequent indications was done by means of anonymised pathology reports. Nevertheless, such data was only available for the year 2019, providing purely a static picture and preventing our analysis from offering a detailed overview on the trends that the different indications have followed during the last decade. The GI system, breast, skin, lung, uterus, ovaries, and prostate are the main body sites on which IHC staining was performed in 2019. From these, GI represented 38.2% of all IHC stains. The two case studies performed in our research focused on the two non-oncologic indications accounting for the highest proportion of all GI IHC stains: *H. pylori* (42%) and coeliac disease (14%%).

A number of points for attention were identified in our analysis. These are mainly derived from our case studies.

Non- invasive versus invasive testing methods

The number of stains are a direct result of the number of gastroscopies performed. In certain circumstances, GI symptoms can be investigated via non-invasive procedures instead of gastroscopies.

Guidelines for the diagnosis of *H. pylori* recommended to prioritise non-invasive testing methods (UBT or stool testing) in the younger adult population, with different age limits that ranged between 45 to 60, in the absence of alarm signs (e.g. weight loss, unexplained anaemia, etc). Our data analysis, show that around 42% of the overall IHC tested population were aged 18-49 and that around 64% of those tested for *H. pylori* in the year 2019 underwent a gastroscopy and invasive testing. Although this appears to be an important proportion of the population, the absence of data on the presence of alarm signs impedes confirmation but calls for some attention to this population segment. Nevertheless, we can observe that the proportion of adults below 50, undergoing a gastroscopy appears to be diminishing slightly overtime, (by 10% between 2012 and 2019).



In coeliac disease diagnosis, all guidelines recommend to first perform a serology test in individuals presenting some common symptoms of coeliac disease or in those recognised as being at high risk of this disease, and only if the blood test proves to be positive, to confirm such positivity via an endoscopy. The value of IHC in this field, appears to be particularly associated to both equivocal cases and refractory coeliac disease and its classification. Although the available reimbursement data allows for an evaluation on the number of serologic tests specifically performed over the study period, and proves for these to have increased overtime, only a small proportion of such tests appear to be closely followed by a gastroscopy (within 3 months). These were assumed to represent positive blood tests in our analysis, given the fact that no data on test results is currently available in the consulted administrative databases. In 76%, of these “positive” cases, at least one IHC stain will be performed. Despite the fact that IHC use remains to this date relatively limited in terms of absolute figures, the number of gastroscopies following serologic tests for coeliac disease has increased in the last decade. This coupled with the fact that the literature indicates a general underdiagnosis in this field and a likelihood for diagnostic testing to grow significantly in the years to come, makes this an interesting area to bear in mind in the future for IHC use. The overall growth of IHC testing in this field grew between 2012 and 2019 by 82%, a higher growth than that of IHC testing in GI (75%) during the same years.

Eradication testing in *H. pylori*

Guidelines on *H. pylori* management, specify that eradication testing should be performed by means of non-invasive tests (i.e. UBT) unless a gastroscopy is required for other reasons, while some respondents to our survey, mentioned that they use IHC for confirming effective eradication of *H. pylori*. Our data analysis also showed that in some cases, treatment for *H. pylori* appears to be followed shortly (within three months) by a gastroscopy. This may represent cases in which invasive testing is performed for eradication checking purposes. However, since no data on indications and diagnosis is available, the extent to which these cases could be representative of patients with other indications requiring a gastroscopy

cannot be elucidated, and represents a further aspect worthwhile exploring in the future.

Routine use of IHC in *H. pylori*

Finally, those guidelines that offered recommendations as to when IHC should be performed for the diagnosis of *H. pylori* highlight the fact that such testing is not required in all cases, and that it should be kept instead for equivocal *H. pylori* diagnoses (following standard histology - H&E). The only exception was the Belgian 2023 consensus study, which recommends routine use of IHC. Similarly, for coeliac disease, current guidelines highlight the added value of IHC in both unclear cases and in those cases in which refractory coeliac disease is suspected.

Whether these recommendations are followed in practice, remains difficult to discern with the limited data currently available, although some respondents to our survey mentioned they routinely use IHC staining specifically for *H. pylori*, primarily due to its high accuracy and in order to avoid missing any positive cases, which could have important consequences in the health of patients, while an effective treatment exists. The analysis of data from pathology reports for the year 2019, showed that *H. pylori* staining was performed in approximately 47% of all gastroscopies. This proportion appears to be higher than the general proportion of IHC staining performed on that same year on regular biopsies (32%). Nevertheless, it indicates that general, laboratories do not perform this stain routinely and that such use is likely to be limited to a small number of laboratories. Indeed our data analysis and the responses received from our survey highlight great variations between laboratories. Such variation may be partly explained by a lack of clear guidelines in this field.

Further limitations linked to our research are worth highlighting:

The registration and the rules to record IHC testing remain difficult to interpret, which may add to the variations in practice registered in the administrative data.



In addition to this, the lack of one source offering an accurate description on the use of these tests, made it necessary to analyse and combine different data sources (i.e. NIHDI / survey from NIHDI / EPS / KCE Limesurvey).

Moreover, the split between the prescriber (most commonly gastroenterologists, followed by gynaecologist, dermatologists, surgeons, and specialist in radio diagnostic) and the decision maker (pathologist, on the basis of the evaluations performed and the results obtained), adds complexity to this field.

Our review of the literature shows the existing guidelines for both *H. pylori* and coeliac disease to rarely mention IHC or go into the specific choice of test, once a gastroscopy has already been performed. Very few do, and those that do, simply present the options. Furthermore, in the review of the guidelines on *H. Pylori*, some inconsistencies were noticed between the Belgian consensus guidelines and the others included in our review, with a recommendation stating the IHC should be performed routinely, but a mention in the document of Giemsa as being an appropriate stain to use. Discussions with experts, highlighted that although Giemsa is indeed an alternative to IHC in *H. pylori* testing, its accuracy is lower than that of IHC, while it takes longer to obtain results, which explains the preference for IHC use. Moreover, no specific additional payment/reimbursement is linked to the use of Giemsa, making it also a less financially attractive test.

Concerning the quality of the guidelines relating to *H. pylori* and coeliac disease identified, it is important to emphasize that the only ones presenting high or intermediate quality, focus on the choice of whether or not to perform a gastroscopy (invasive method or non-invasive). The few guidelines covering the specific research question on whether to perform IHC testing are of poor quality (according to the AGREE tool).

General EM use in Belgium

EM investigation is currently performed in few centres in Belgium and for a limited number of indications. Kidney biopsies were by far the most commonly analysed specimens via EM. Seven out of the nine laboratories with EM capacity carried out this type of investigation. The second and third most frequently analysed tissues were skin, and ENT (mostly cilia) although these analyses were almost exclusively carried out in a single laboratory. Muscle biopsies were the fourth most common, and were carried out by six laboratories.

Our literature review yielded few high-quality studies that made specific recommendations on the most appropriate diagnostic techniques for the groups of diseases studied. The sparse published literature on recommended diagnostic methods, together with a lack of data on the total number of cases investigated for these diseases, means that we do not have sufficient information to conclude to what extent the current use of EM is appropriate.

According to the gathered evidence, EM is one of the standard and appropriate techniques used to diagnose glomerular and other kidney diseases (e.g. TMA in the kidney allograft), although we have not found any guidelines specifying whether EM should routinely be used on all kidney biopsies or whether there are specific instances in which it should not be used. According to the (non-systematic) review concerning IgM nephropathy, EM is an optional diagnostic method when this condition is suspected. It is important to highlight that literature review did not cover the full spectrum of potential pathologies that can be investigated on kidney biopsies. Indeed, a full list of evidence-based indications for carrying out a kidney biopsy would be needed.

Concerning muscle pathology, we were also unable to identify high-quality studies (guidelines or systematic reviews) that could provide evidence and guidance as to the most adequate diagnostic techniques in specific situations. According to the articles reviewed, the use of EM is considered valuable or even essential when genetic testing (when applicable) is inconclusive or other pathology techniques (e.g. LM) cannot provide an answer.



Concerning the EM examination of cilia, we found high-quality (European and North American) guidelines that consider TEM to be part of the diagnostic pathway to diagnose PCD, although it is not the recommended first-line investigation. Whether the current use of EM for PCD diagnosis corresponds to this second-line investigation cannot be inferred from our analysis.

In the case of EDS diagnosis, the frequent skin biopsies investigated for EDS by a single laboratory in Belgium raises some questions. Two studies provided evidence supporting genetic testing as the gold-standard investigation for all suspected EDS subtypes (with the exception of hypermobile EDS). Two specific EDS subtypes can additionally be investigate by urinalysis. The authors state that only when genetic testing does not yield clear or concluding results, TEM can be useful in supporting, although not confirming, a EDS diagnosis. The articles also state that when hypermobile EDS is suspected, this should be diagnosed clinically. The current investigations of skin biopsies for EDS by a single laboratory in Belgium may possibly correspond to the centralised analyses for all national cases that could not be diagnosed on a genetic basis alone, or they may correspond to an over-use of EM instead of genetic testing (or clinical diagnosis for hEDS). To answer the questions, further exploration into these investigations is warranted.

Electron microscopy is a technique that's relatively expensive but which can be extremely informative in specific cases when adequately used. Various articles stated the importance of analysing biopsies with EM in specialised centres and by specialised pathologists. In Belgium there exists a system of neuromuscular centres for instance, that can help in leading the diagnosis and the therapy in a scientific way. Because the disorders concerned are often rare disorders, centralising the diagnosis of these disorders is indeed important.

It is important to emphasise that we did not identify relevant literature addressing the most appropriate diagnostic techniques for specific pathologies for which biopsies are often taken, such as specific renal diseases and neuromuscular pathologies not specifically covered in our literature, likely because they are rare. Consequently, we cannot draw conclusions for the unexplored pathologies and must bear in mind that the absence of evidence does not imply an absence of EM usefulness in fields that were not covered.

One propose alternative technique is genetic testing. In our data analysis, it was not possible to identify genetic testing specific for the indications for which EM is used. Moreover, using the EPS data would have been inappropriate because too few EM investigations are done for such a source to be representative.

The research presented here highlights important data gaps, (absence of indications or test results in the Permanent Sample (EPS), availability of data from pathology reports containing details on IHC staining limited to the year 2019, anonymous voluntary survey of laboratories capturing self-reported responses), which impede to obtain a clear picture and analysis on the use of the studied tests. An effort should be done to ensure such gaps are addressed on an appropriate way, increasing transparency, while avoiding too much complexity. This should support future informed decision making in a field that could benefit from some standardisation, given the great variations in practices it presents.



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