

REVIEW – DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

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Abstract – In this review, we present an overview of the main articles published in the field of *Helicobacter pylori* diagnosis in the past year. Key themes emerging in this area include rapid point-of-care testing and the use of artificial intelligence to enhance the efficacy of existing invasive diagnostic platforms. Studies in the field of endoscopy focussed on improving detection methods to diagnose *H. pylori* and associated diseases, targeted biopsy sampling, gastric juice analysis by Endofaster and the adoption of artificial intelligence in medical imaging. There have been some studies evaluating staining protocols for histopathology and exciting developments in the field of digital pathology for *H. pylori* detection and characterisation of inflammatory pathologies. Many articles have addressed the need for rapid, accurate, non-invasive point-of-care tests to analyse saliva or stool for the detection of *H. pylori* and/or its antimicrobial resistance. Finally, developments and considerations for the molecular detection of *H. pylori* and antimicrobial resistance using gastric samples are also discussed.

Keywords: Invasive, Non-invasive, Endoscopy, Histopathology, Artificial intelligence, Molecular test, Point-of-care test, Antimicrobial susceptibility testing.

INVASIVE DIAGNOSIS

Endoscopy

Endoscopic diagnosis of *Helicobacter pylori* and related diseases has seen ongoing enhancements through improved imaging and detection techniques. In the past year, Kitagawa et al¹ employed texture and colour enhancement imaging (TXI) to improve the accuracy of endoscopic diagnosis of active *H. pylori* gastritis based on the Kyoto Classification of Gastritis². The study revealed that TXI was more efficient for visualizing gastric mucosal atrophy and accurately detecting diffuse redness, which are key indicators of active *H. pylori* infection. Moreover, the study showed better inter-observer agreement among reviewers using TXI than using white light imaging, emphasizing the reliability of TXI for diagnosing *H. pylori* infection¹.

Yada et al³ evaluated the relationship between the scratch sign (a red linear, scrape-like appearance with white deposits that may be present on the gastric mucosa at the lower greater curvature of the gastric body) and *H. pylori*, reporting the scratch sign as a useful endoscopic predictor of an *H. pylori*-negative gastric mucosa. However, the small sample size and a limited number of endoscopists examining the patients highlighted the need for more extensive, prospective clinical studies to further establish the utility of the scratch sign³.

I-scan optical enhancement (OE) has been suggested to distinguish areas of the infected mucosa for targeted biopsy sampling. Dawod et al⁴ conducted a prospective study to evaluate the effect of this I-scan OE technology on the diagnostic yield of the Campylobacter-like organism



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(CLO) test. They found that targeted sampling for the CLO-test, with the aid of the I-scan OE, significantly shortened the reading time of positive results with high test sensitivity⁴, thus facilitating early diagnosis and timely management of *H. pylori* infection.

Since *H. pylori* resides in the mucus layer, Soh et al⁵ compared the diagnostic accuracy of the rapid urease test (RUT) and bacterial load of *H. pylori* between gastric mucosal swabs and biopsies. Overall, the swabs showed higher RUT accuracy and bacterial load than tissue biopsy, with sensitivity and specificity values of 98% and 100%, respectively, for the swab RUT⁵.

Endofaster (Niso Biomed Srl, Torino, Italy) is a device that detects the presence of *H. pylori* by determining the ammonium concentration of gastric juice in real-time during endoscopy⁶. A number of studies evaluating Endofaster have been published in the past year. Firstly, the diagnostic accuracy of Endofaster versus histology was prospectively evaluated by Zullo et al⁷ across 12 endoscopic units in Italy, with sensitivity and specificity values of 86.3% and 83.3%, respectively (Table 1). The same group also reported that Endofaster improves *H. pylori* detection in cases of chronic active gastritis, without clear presence of *H. pylori* at standard histological examination.⁸ Thirdly, this team reported that Endofaster may be useful in ruling out atrophic gastritis⁹. In all 3 studies, concurrent proton pump inhibitor use and previous *H. pylori* eradication did not affect the test accuracy⁷⁻⁹. Finally, Vasapoli et al¹⁰ reported high diagnostic performance of Endofaster in the real-life clinical setting (N=198), with sensitivity, specificity, positive predictive and negative predictive values of 91.5%, 93%, 84.3% and 96.4%, respectively.

HISTOPATHOLOGY

In an effort to improve the diagnostic performance of histological diagnosis, Chu et al¹¹ evaluated a modified silver staining protocol for the detection of *H. pylori*. Gastric antrum and gastric angle mucosal biopsies from 60 patients were analysed using an *H. pylori* immunohistological assay, a methylene blue histochemical assay and modified silver nitrate staining compared to the ¹⁴C-urea breath test (UBT). The highest accuracy was achieved using the modified silver staining protocol¹¹.

Khan et al¹² evaluated Hematoxylin and Eosin (H&E), Giemsa and modified toluidine blue (MTB) in comparison to immunohistochemistry for *H. pylori* detection using gastric biopsy specimens from 50 patients (Table 1). The lowest sensitivity was observed for H&E (46.8%),¹² suggesting that the use of either Giemsa or MTB are more reliable alternatives to H&E, in particular in cases where more expensive immunohistochemistry is not available.

NON-INVASIVE DIAGNOSIS

Serology

While unsuitable for post-eradication testing, *H. pylori* detection by serology is a quick and inexpensive method for diagnosing *H. pylori* when adequate diagnostic facilities are not available and for *H. pylori* screening in populations at high risk of gastric cancer. Due to variations in the accuracy of serological tests, they should be validated in the target population before use. *H. pylori* serology-related publications in the past year have focussed on increasing speed and ease of use, with a view to providing an option for point-of-care diagnosis. Firstly, a rapid thin-layer immunoassay to detect *H. pylori* in serum of gastritis and ulcer patients compared to ELISA demonstrated very rapid time to obtain results (within minutes) and was inexpensive¹³. However, the sensitivity of the assay (Table 1) needs further optimisation. Indeed, a rapid point-of-care test (POCT) for *H. pylori* serology has been lacking to date in terms of accuracy. However, Schulz et al¹⁴ described a new *H. pylori* lateral flow POCT that detects the flagellar filament capping protein (FlaB) and the cytotoxin-associated gene A (CagA). The assay demonstrated excellent sensitivity and very good specificity (Table 1) using either whole blood or serum samples when compared to histology and culture¹⁴. Further evaluation in different populations is warranted.

Functional serology to investigate gastrin-17 and pepsinogen levels can provide valuable clinical information on gastric atrophy and may identify those at higher risk of gastric cancer¹⁵. Jeong et al¹⁶ characterised pepsinogen assay findings indicative of ongoing *H. pylori* infection (as determined by the ¹³C-UBT) and reported that serum pepsinogen II levels >12.95 ng/mL and pepsinogen-I/

TABLE 1. SENSITIVITY AND SPECIFICITY OF TESTS DESCRIBED IN THIS REVIEW.

Test	Sample	Diagnosis	N	Sensitivity	Specificity	Reference method
Endofaster ⁷	Gastric juice	<i>H. pylori</i>	1138	86.3%	83.3%	Histology
H&E staining ¹²	Biopsies	<i>H. pylori</i>	20	46.8%	88.8%	IHC
Giemsa staining ¹²	Biopsies	<i>H. pylori</i>	20	90.6%	88.8%	IHC
MTB staining ¹²	Biopsies	<i>H. pylori</i>	20	93.7%	83.3%	IHC
Thin layer immunoassay ¹³	Serum	<i>H. pylori</i>	214	67-72%	100%	ELISA
Dual FliD and CagA POCT ¹⁴	Whole blood and serum	<i>H. pylori</i>	111	98.1-100%	89.1%-94.3%	Histology and culture
HpSA-LFIC ¹⁹	Stool	<i>H. pylori</i>	200	93.8%	59.8%	ELISA
SD Biotec <i>H. pylori</i> SAT ²¹	Stool	<i>H. pylori</i>	150	85.1%	97.6%	PCR
Solid scintillation 14C-UBT ²²	Breath	<i>H. pylori</i>	239	95.4%	97.5%	RUT and histology
RIDA® GENE <i>H. pylori</i> ³²	Biopsy	<i>H. pylori</i> ClarR	120	100% 97.1%	99.1% 100%	In-house PCR
Taqman-MGB probe multiplex real-time PCR ³⁵	Biopsy	<i>H. pylori</i> ClarR LevoR	697	100% 100% 100%	97.9% 95.6% 100%	Sequencing
Duplex RAA-LFD ³⁶	Clinical <i>H. pylori</i> isolates	cag-A-positive <i>H. pylori</i>	56	96%	100%	Duplex PCR
Smart Gene™ assay ³⁸	Gastric juice	<i>H. pylori</i> ClarR	Various 77	92.8% 100% 98.4% 95.0% 91.7%	94.4% 95.9% 88.9% 98.6% 100%	UBT Culture SAT PCR Culture
Nested PCR ⁴²	Oral mucosa	<i>H. pylori</i>	41	92.3%	98%	RUT
PyloriDx ⁴⁶	Stool	<i>H. pylori</i>	254	98.5%	98.9%	SAT

H&E: hematoxylin and eosin; IHC: immunohistochemistry; MTB: modified toluidine blue; ELISA: enzyme-linked immunosorbent assay; FliD: flagellar filament capping protein; CagA: cytotoxin-associated gene A; POCT: point-of-care test; HpSA-LFIC: *H. pylori* polyclonal stool antigen lateral flow immunochromatography assay; SAT: stool antigen test; PCR: polymerase chain reaction; UBT: urea breath test; RUT: rapid urease test; ClarR: clarithromycin resistance; LevoR: levofloxacin resistance; RAA-LFD: recombinase-aided amplification combined with lateral flow dipstick.

II ratios of <4.35 suggest ongoing infection in asymptomatic individuals. Zhou et al¹⁷ investigated the impact of *H. pylori* infection (as determined by ¹⁴C-UBT) on serum levels of both pepsinogens and gastrin-17. A total of 354,972 participants attending health checks were recruited. Mean levels of pepsinogens and gastrin-17 were higher, while the mean ratio of pepsinogen-I/II was lower in *H. pylori*-infected versus uninfected individuals¹⁷. Moreover, among *H. pylori*-positive individuals, pepsinogen and gastrin-17 levels correlated positively with the UBT value, while the ratio of pepsinogen-I/II correlated negatively with the UBT value, suggesting that *H. pylori*-positive patients with a higher UBT value are unlikely to have gastric atrophy but may have a greater risk of severe gastritis or peptic ulcers¹⁷.

THE STOOL ANTIGEN TEST AND UREA BREATH TEST

With regards to the stool antigen test (SAT), Resina et al¹⁸ carried out a prospective multicentre study in Spain (9 centres, N=307) to evaluate the diagnostic performance of the automated LIAISON[®] Meridian *H. pylori* monoclonal antibody SAT compared to the ¹³C-UBT. They reported sensitivity and specificity values of 67% and 97%, respectively¹⁸. Stool DNA was analysed for *H. pylori* in the discordant samples and the SAT accuracy reanalysed, resulting in sensitivity and specificity of 94% and 97%, respectively, suggesting the LIAISON Meridian assay to be a good option for SAT¹⁸.

Abdelmalek et al¹⁹ investigated a *H. pylori* polyclonal stool antigen lateral flow immunochromatography assay (HpSA-LFIC) compared to a monoclonal antibody-based ELISA kit from 200 study participants, demonstrating that the HpSA-LFIC had high sensitivity (93.8%; Table 1) and negative predictive value (98%), but low specificity, positive predictive value and accuracy (59.8%, 31.3% and 65.3%, respectively). The assay is currently not accurate enough to recommend its use for the sole diagnosis of *H. pylori* infection. Using stool samples from 141 students, Kakiuchi et al²⁰ reported that the bioluminescent enzyme immunoassay (B[EIA]; Eiken) is superior to other SATs currently on the market in Japan. Additionally, a Ugandan study²¹ involving 150 dyspeptic patients, demonstrated good diagnostic accuracy for the SD Biotec[™] *H. pylori* antigen testing kit (Standard Diagnostic Inc.) compared to PCR (Table 1).

In terms of non-invasive diagnosis by means of the UBT, an open-label prospective multi-centre study conducted by Han et al²² reported high diagnostic value for a novel solid scintillation ¹⁴C-UBT in diagnosing *H. pylori* compared to the RUT and histology in 239 participants in China (Table 1).

ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

Artificial Intelligence & Endoscopy

One of the significant advances in the detection of *H. pylori* has been the evaluation of artificial intelligence (AI) and machine learning techniques in medical imaging. In the past year, many studies in this research area have originated in Asia. Zhang et al²³ developed an explainable AI system for the diagnosis of *H. pylori* infection (named as EADHI) that auto-identifies *H. pylori* infection through the recognition of multiple mucosal features by endoscopy. Interestingly, EADHI demonstrated superior diagnostic ability compared to endoscopists, showing promise for assisting in clinical screening for *H. pylori* infection. The diagnostic logic of EADHI closely mirrored that of endoscopists, potentially enhancing its trust and acceptability among clinicians. However, limitations such as retrospective data collection from a single centre and lack of prospective validation were identified, necessitating further research to validate and globally apply this AI-based diagnostic system²³.

Li et al²⁴ introduced and evaluated another deep learning-based system named Intelligent Detection Endoscopic Assistant-*Helicobacter pylori* (IDEA-HP) to detect *H. pylori* infection using endoscopic videos in real-time. Trained on an extensive dataset of endoscopic images and videos, IDEA-HP matched the overall accuracy of expert assessments, considerably outperforming beginners in diagnostic accuracy and sensitivity. IDEA-HP demonstrated promising potential for assisting endoscopists in assessing *H. pylori* infection status in real-time during the performance of clinical examination²⁴.

Lastly, Seo et al²⁵ developed and validated a convolutional neural network (CNN) model for diagnosing *H. pylori* infections using endoscopic images. Despite various ethnicities in the internal and external validation datasets, the CNN model demonstrated a high degree of sensitivity, specificity and accuracy in predicting *H. pylori* infection status²⁵.

ARTIFICIAL INTELLIGENCE & HISTOPATHOLOGY

Advancements in AI and digital pathology may transform the process of diagnosing *H. pylori* and related inflammatory pathologies. One of these significant transformations comes in the form of high-resolution digitalization, which is crucial for the accurate detection of *H. pylori* in virtual slides. Uguen et al²⁶ demonstrated that high-resolution digitalization (40X magnification) had very good concordance in diagnosing *H. pylori* compared to traditional glass slides. In another study, the use of AI with digital pathology demonstrated a high level of accuracy for *H. pylori* diagnosis at a lower magnification (20X magnification) when the Warthin-Starry silver stain was used²⁷. AI's capacity to recognize subtle morphological changes has led to the development of a method for identifying the coccoid form of *H. pylori*, a significant cause of refractory *H. pylori* infection. Manual recognition of coccoid *H. pylori* on histopathology slides is time-consuming, labour-intensive, and often over-looked²⁸. Zhong et al²⁸ evaluated the YOLO v5 CNN model for the detection of coccoid *H. pylori* and their method achieved a mean absolute error equivalent to that of a senior pathologist. These findings highlight AI's potential to match the diagnostic accuracy of experienced professionals and pave the way for more efficient treatment options.

Coupled with the right data and training images, deep learning algorithms can provide a powerful tool to distinguish between *H. pylori*-associated inflammatory pathologies. The DeepHP database, a publicly curated dataset containing over 394,000 histopathological images, was presented as a valuable resource for the community²⁹. This database enables researchers and pathologists to train convolutional neural networks (CNNs), enhancing the accuracy of diagnosing *H. pylori*-related conditions. Experiments conducted using DeepHP and three different CNN models have demonstrated impressive results, showcasing the potential of AI in computational pathology²⁹. Indeed, another recent study demonstrated that a deep learning CNN could accurately distinguish between *H. pylori* gastritis and autoimmune gastritis, achieving results comparable to expert gastrointestinal pathologists³⁰. Similarly, a method proposed by Yacob et al³¹ used an improved deep CNN with pooling and a canonical correlation analysis feature fusion method to accurately detect *H. pylori*-related atrophic gastritis. This hybrid model achieved an accuracy of 98.2%³¹.

MOLECULAR DETECTION OF *H. PYLORI* AND ANTIMICROBIAL RESISTANCE

Invasive Molecular Tests

Time and ease-of-use are critical in the clinical laboratory, so complex molecular testing must be optimised efficiently. A French team used the BD MAX™ system (Becton Dickinson) to automate biopsy DNA extraction and PCR amplification using the commercially available RIDA®GENE *H. pylori* kit (R-Biopharm, Darmstadt, Germany)³². After initially optimising the method using 31 biopsies, the method was further tested prospectively on 210 biopsies and compared to in-house PCR. The platform demonstrated excellent sensitivity and specificity, both for the detection of *H. pylori* and clarithromycin-resistance mediating mutations³² (Table 1).

Haumaier et al³³ developed a method of detecting quinolone resistance mutations in the *gyrA* gene, with a view to providing a more rapid option to guide second-line treatment if first-line treatment fails. Using FRET-based quantitative PCR (qPCR) with a Cy5-labelled probe means this can be done on most qPCR machines. A total of 8 patient biopsy samples were tested using this method, then confirmed by sequencing showing its efficacy³³.

Luan et al³⁴ investigated accelerated cycling PCR (AC-PCR) as a method to more accurately and swiftly detect single-nucleotide DNA mutations associated with clarithromycin resistance. AC-PCR relies on a specific primer design, fast DNA polymerase, and faster cycling times to yield

results within 30 minutes. When tested on biopsy samples from *H. pylori*-infected patients (N=50) and compared to sequencing data, an agreement of 98% (49/50) for the detection of clarithromycin resistance was reported³⁴.

Zhao et al³⁵ established a Taqman-MGB probe multiplex real-time PCR system for the detection of *H. pylori* and both its clarithromycin and levofloxacin resistance, demonstrating excellent sensitivity and specificity (Table 1) in the analysis of biopsy tissue DNA compared to sequencing, while Zhu et al³⁶ demonstrated very good accuracy (Table 1) with their duplex recombinase-aided amplification combined with lateral flow dipstick (Duplex RAA-LFD) for the detection of *cagA*-positive *H. pylori* DNA from clinical isolates compared to PCR.

Wang et al³⁷ developed a rapid visual assay based on loop-mediated isothermal amplification (LAMP) for the detection of *H. pylori* and its virulence factor genes *cagA* and *vacA*. The assay was fast (30 minutes), low-cost, and enabled sensitive DNA amplification (10^{-3} ng/ μ l) from biopsy samples without the need for expensive equipment or highly trained staff. A novel Smart GeneTM (Mizuho Medy Co., Ltd) *H. pylori* molecular POCT was evaluated in its ability to detect *H. pylori* and clarithromycin resistance from patient intragastric fluid. For intragastric fluid analysis, results were compared to the UBT, SAT, PCR and culture, with culture showing the highest accuracy for *H. pylori* diagnosis³⁸ (Table 1). The assay also showed high accuracy for the detection of clarithromycin resistance compared to culture-based antimicrobial susceptibility testing (AST)³⁸ (Table 1).

Two studies in the last year have highlighted the importance of primer choice and design in PCR-based molecular *H. pylori* diagnostics. Firstly, variation in the detection of *H. pylori* using different primers has been demonstrated by Elnosh et al³⁹ PCR targeting the *16S rRNA* gene exhibited the best results for molecular detection of *H. pylori* in DNA isolated from biopsy samples (N=290) compared to other genes (*ureA* and *glmM*). Further, laboratory evaluation of in silico-designed primers was recommended by Abdelmalek et al⁴⁰

With regards to DNA sequencing, a Chinese study from Beijing⁴¹ used Sanger sequencing to characterise resistance mechanisms in strains that were initially characterised by culture and AST (Etest): 180 clinical strains were investigated for 5 genetic antimicrobial resistance determinants (mutations in the *23S rRNA*, *pbp1*, *rdxA*, *gyrA* and *gyrB* genes). The results showed congruency between molecular and culture-based methods⁴¹.

NON-INVASIVE MOLECULAR TESTS

Research from Jara et al⁴² investigated the feasibility of a non-invasive PCR-based test for oral sample analysis. In their study, oral mucosa swabs and saliva were collected from patients prior to endoscopy, DNA was isolated, and a nested-PCR was carried out. There was congruency between RUT and oral mucosa results, but not for saliva PCR and the RUT or for oral mucosa and saliva PCR⁴². The sensitivity and specificity values obtained for oral mucosa PCR compared to the RUT for *H. pylori* diagnosis are shown in Table 1.

Mei et al⁴³ evaluated a POCT approach for the detection of *H. pylori* in saliva. They used a paper-based nucleic acid enrichment method based on lateral flow, which was portable and required nothing but a small battery-powered reading device, making it ideal for field testing. Based on recombinase polymerase amplification to detect the *H. pylori ureB* gene, this test could generate results in 30 minutes from contrived *H. pylori*-positive saliva samples with a sensitivity similar to that of PCR⁴³. Developed alongside this test was a smartphone application that could mitigate a user's visual bias or inability. This application could use the camera's image to compare pixel intensity between sample and control to give accurate unbiased results⁴³. This novel machine and test show good promise but need further diagnostic accuracy evaluation using saliva samples from patients with confirmed *H. pylori* status.

A small study from Japan investigated the relatedness and phylogeny of 21 individual patients with gastric cancer and positive SATs. Saliva, dental biofilm, and gastric biopsies underwent multi-locus sequence typing using 8 housekeeping genes⁴⁴. Of these, only one patient had matching oral-gastric *H. pylori* genotypes, six strains showed phylogenetic relatedness but were not from the same origin⁴⁴. No culture-based analysis took place to investigate phenotypic relatedness. The possibility of different strain colonisation in the oral versus gastric environment is worth considering for saliva-based tests, especially if saliva DNA is subjected

to molecular AST. Further research into the relationship between oral and gastric colonisation is needed to fully establish whether these differences in genotype between oral and gastric genotypes are common.

For stool sample analysis, the Smart Gene™ POCT assay that was tested using intragastric fluid samples (as described in Section 4.1 above), has also been evaluated as a non-invasive POCT for the diagnosis of *H. pylori* and clarithromycin resistance using stool⁴⁵. Stool samples from 139 patients that tested positive by SAT were included in the analysis.⁴⁵ The *H. pylori* detection rate was 95.7% using the Smart Gene™ assay, 92.8% using real-time PCR and 89.2% by sequencing analysis⁴⁵. The concordance between the Smart Gene™ assay and sequencing for the detection of clarithromycin resistance mutations was 96.7%⁴⁵.

Molecular-based next generation sequencing (NGS) is now available to patients in some parts of the world, such as the USA and provides AST based on the detection of *H. pylori* DNA mutations associated with phenotypic resistance⁴⁶. Advantages include detecting resistance to multiple antibiotics simultaneously and quickly. NGS has been reported to produce AST results comparable to culture-based methods from biopsy tissue.⁴⁷ In a key first-of-its-kind multi-centre study, AMR-associated DNA mutations from stool samples were analysed by sequencing and compared to paired gastric biopsy sample results of the same patients in the USA. Firstly, the authors demonstrated excellent accuracy of the PyloriDx stool PCR assay (American Molecular Laboratories, Vernon Hills, IL, USA) compared to the SAT for the detection of *H. pylori*⁴⁶ (Table 1). The results from the stool-sequenced DNA correlated closely to those from the biopsy DNA (N=70) and were concordant in 91.4% of cases.

CONCLUSIONS

International consensus guidelines published in the last year highlight the importance of accurate testing in the appropriate management of *H. pylori*^{15,48-51}. There has been continued research and productivity into improved detection methods for diagnosing infection, characterising *H. pylori*-driven pathologies and detecting antimicrobial resistant strains. Advancements in detection methods leveraging AI, machine learning, and advanced endoscopic techniques are contributing significantly to improving the accuracy, speed, and efficiency of *H. pylori* diagnosis. Along with the right data and training images and extensive validation in different populations, AI is likely to play an increasingly central role in the field of endoscopy. The integration of digital pathology and AI shows enormous promise for histological diagnosis. With high-resolution digitalization, reliable data sets, sophisticated deep learning algorithms, and robust AI models, the diagnosis and treatment of *H. pylori*-related conditions could be significantly enhanced. Furthermore, advances in the development of more accurate POCTs to diagnose *H. pylori* and/or its resistance to antimicrobials using serum, stool and gastric sample analysis show potential for more widespread incorporation of these approaches into clinical use.

Conflict of Interest

The authors declare no conflict of interest.

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Informed Consent

Informed consent was not required for this review article.

Authors' Contributions

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