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# Atypical Lymphoid Proliferations and Clonality in *Helicobacter*-associated Inflammatory Infiltrates in Children

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# Extranodal marginal zone lymphoma of mucosa—associated lymphoid tissue (MALT lymphoma)

- Extranodal lymphoma
- Morphologically heterogeneous small B cells
  - Marginal zone (centrocyte—like) cells,cells resembling monocytoid cells， small lymphocytes，scattered immunoblasts， centroblast—like cells，plasmacytic differentiation in some cases
- Marginal zones of reactive B-cell follicles ， interfollicular region，follicles (follicular colonization)
- Lymphoepithelial lesions

# Extranodal marginal zone lymphoma of mucosa—associated lymphoid tissue (MALT lymphoma)

- ICD-O code 9699/3
- Accounts for 7—8% of all B-cell lymphomas and for as many as 50% of primary gastric lymphomas
- Most cases occur in adults， median patient age in the seventh decade of life

# Extranodal marginal zone lymphoma of mucosa—associated lymphoid tissue (MALT lymphoma)

- Immunohistochemistry
  - CD20+, CD79a+, CD5–, CD10–, CD23–, CD43+/–,
  - Infrequent cases are CD5+,very rare cases are CD10+ but BCL6–
  - CD21, CD23, and CD35 typically reveals expanded meshworks of follicular dendritic cells
- The demonstration of light chain restriction is helpful in the differential diagnosis with reactive hyperplasia
- The tumour cells of MALT lymphoma typically express IgM heavy chains, and less often IgA or IgG
- Natural course and are slow to disseminate

# Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

- Occur in patients older than 50 years ,extremely rare in the pediatric population
- Association with chronic H pylori,less prevalent Helicobacter heilmannii
- Standard first-line treatment for Helicobacter-associated primary gastric MALT lymphoma is the eradication of Helicobacter using triple therapy
- Almost 10% of MALT lymphomas are unrelated to H pylori infection, and the pathogenesis remains unclear
  - Genetic alterations (t(11;18)) and the *NF-kB* activation
  - Infections associated with C jejuni

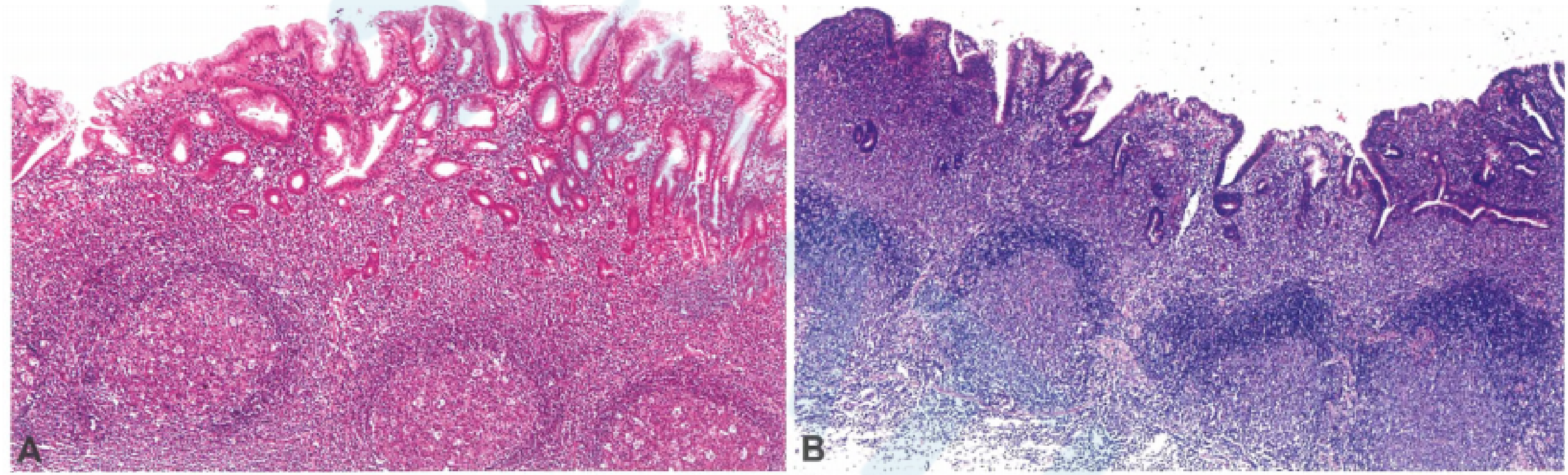



**Fig. 13.58** Gastric MALT lymphoma. **A** The tumour cells surround reactive follicles and infiltrate the mucosa. The follicles have a typical starry-sky appearance. **B** The marginal zone cells infiltrate the lamina propria in a diffuse pattern and have colonized the germinal centres of reactive B-cell follicles. The colonized follicles do not show a starry-sky pattern.

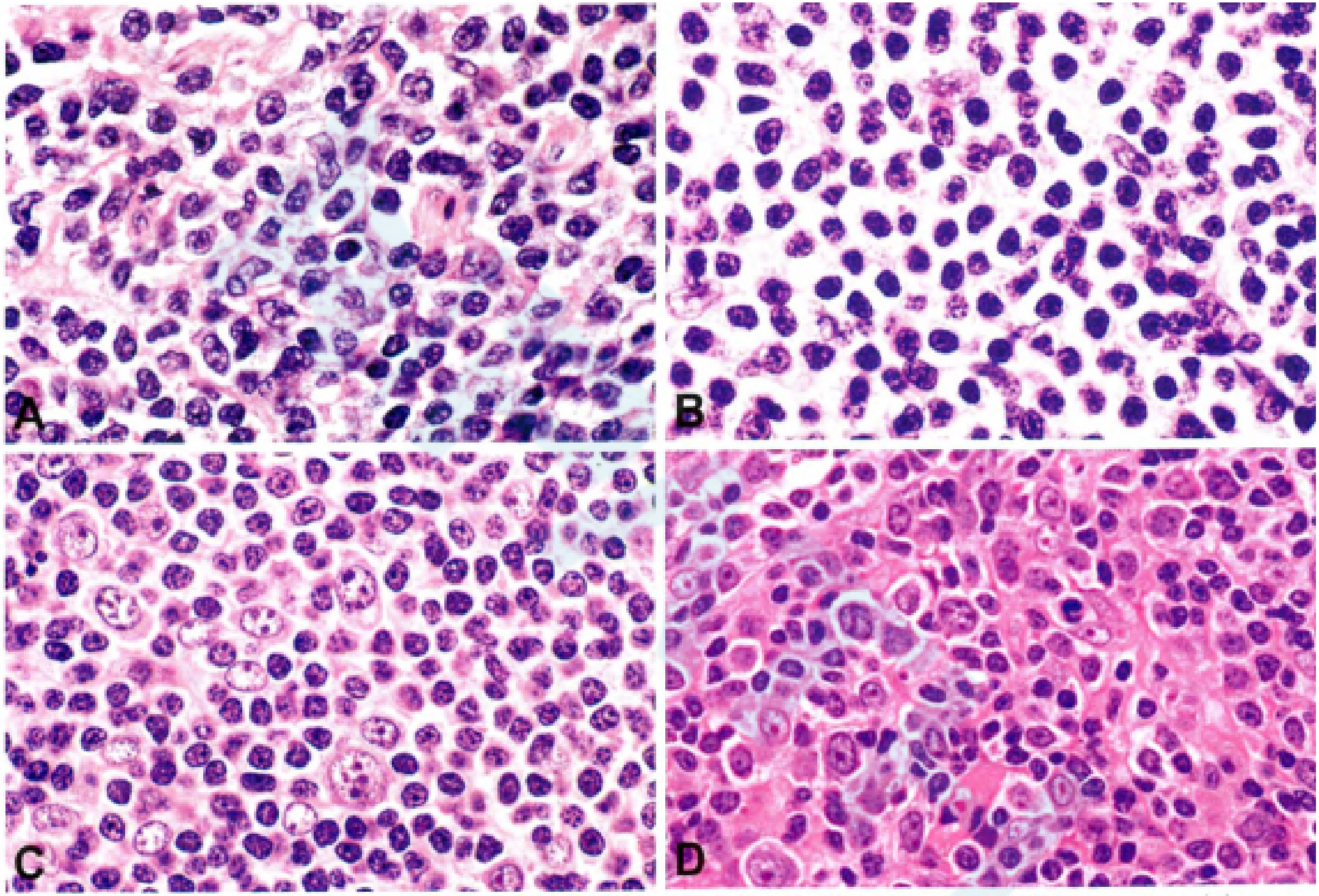



**Fig. 13.60** Morphological spectrum of MALT lymphoma cells. **A** Neoplastic marginal zone B cells with nuclei resembling those of centrocytes, but with more-abundant cytoplasm. **B** The cells of this MALT lymphoma have abundant pale-staining cytoplasm, resulting in a monocytoid appearance. **C** Lymphoma cells resembling small lymphocytes. There are scattered transformed cells. **D** Increased number of large cells.

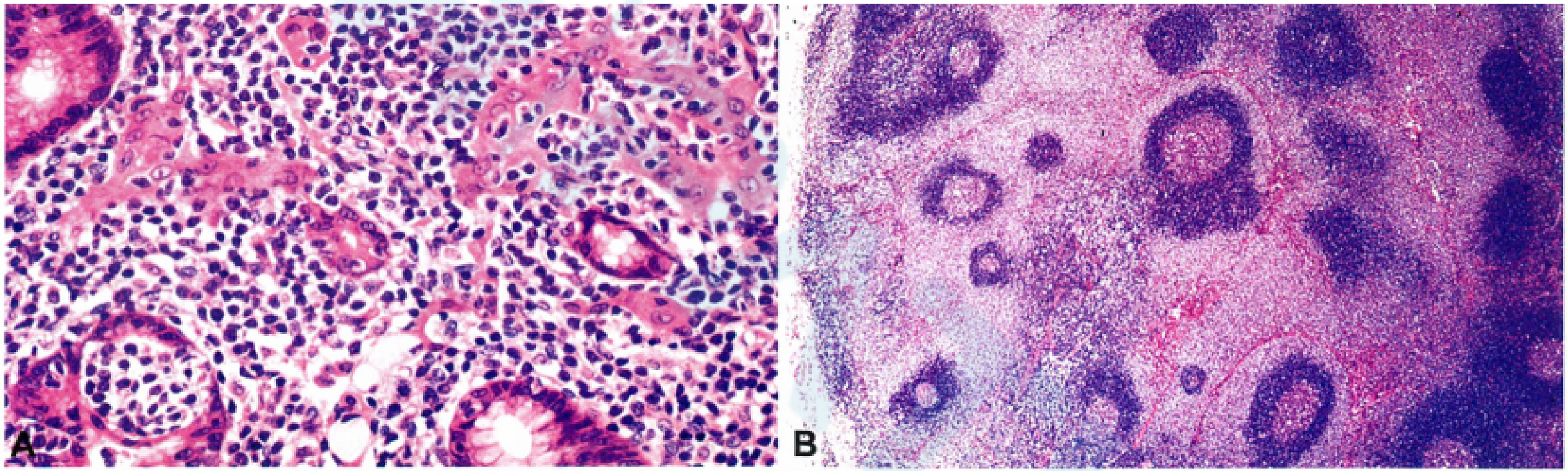
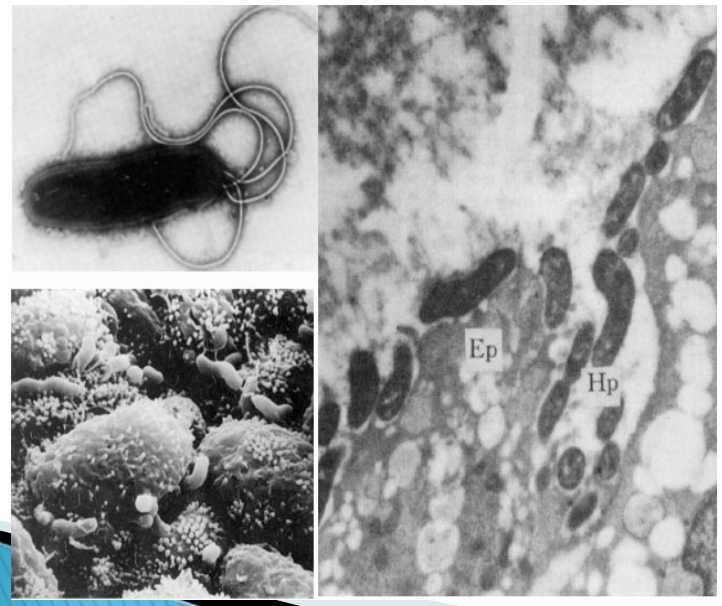
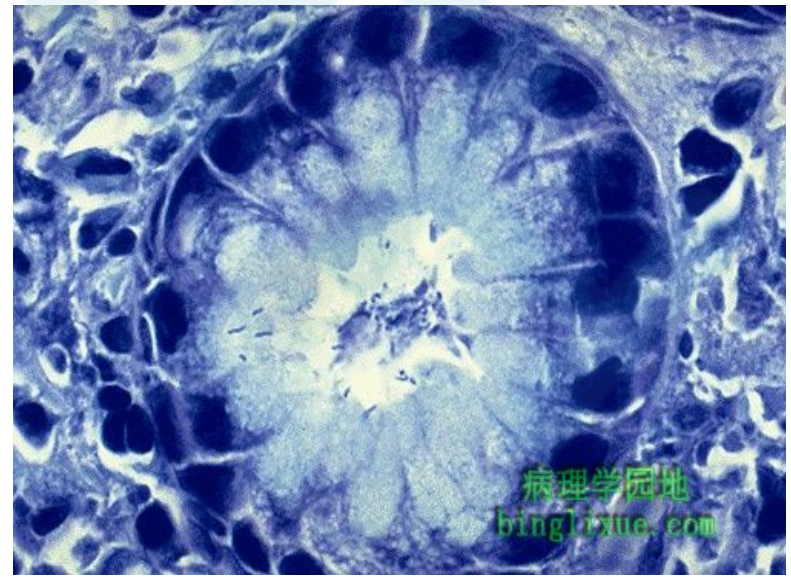



**Fig. 13.59** MALT lymphoma. **A** Gastric MALT lymphoma with prominent lymphoepithelial lesions. **B** Gastric lymph node involved by MALT lymphoma. The tumour cells infiltrate the marginal zones and spread into the interfollicular areas.

# Helicobacter pylori

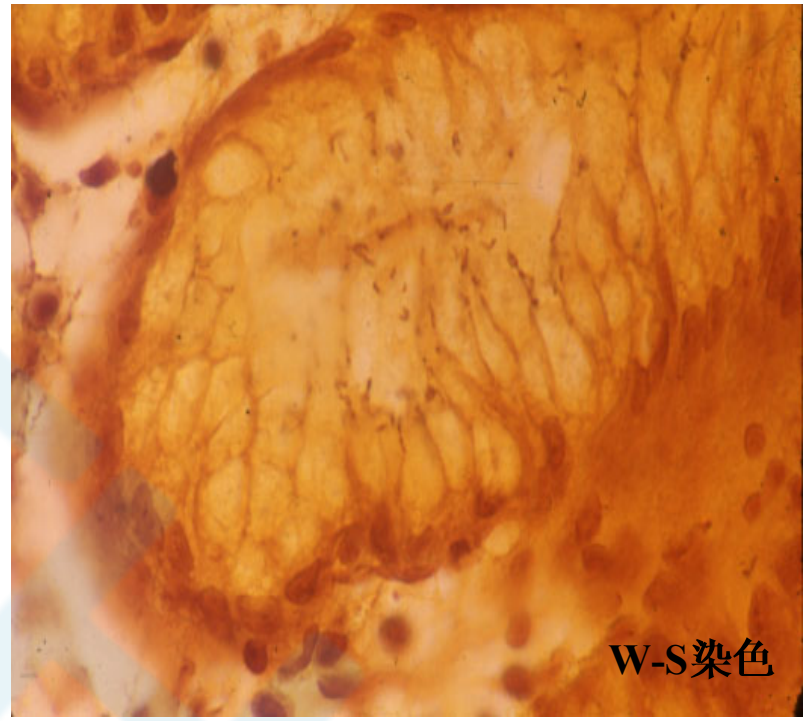
- One of the most common pathogenic infections in humans, with prevalence rates reaching 60% in adults
- A spiral-shaped bacterium
- Can be seen in hematoxylin and eosin (H&E) staining
  - Sensitivity and specificity of H&E stain has been reported as 69-93% and 87-90%, respectively



# Helicobacter pylori

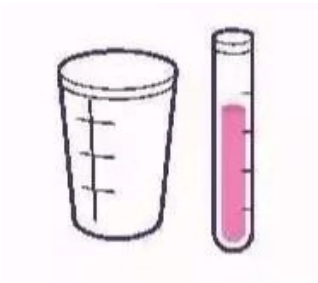
- Modified Giemsa stain, Warthin-Starry silver stain, Genta stain, and immunohistochemical (IHC) stain
  - The specificity can be improved 90-100%



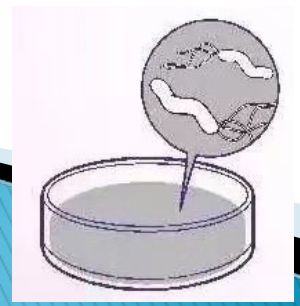

- H. pylori infection
  - Chronic gastritis
  - Peptic ulcer disease
  - Gastric adenocarcinoma
  - Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

# Helicobacter pylori

- Several diagnostic methods have been developed for the aim of accurate detection of this organism
- Noninvasive method
  - Serology, urea breath test, or stool antigen test



- Invasive methods
  - Culture, histological examination, and rapid urease test



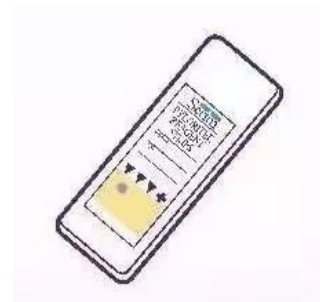


**Table 1** Diagnostic tests for the detection of *H. pylori* infection (2,15-17)

| Test | Sensitivity | Specificity | Advantages | Disadvantages |
|---|---|---|---|---|
| Noninvasive | | | | |
| Serology | 76-84 | 79-90 | Widely available, inexpensive | Positive result may reflect previous rather than current infection, not useful after treatment |
| Urea breath test | >95 | >95 | High negative and positive predictive values, useful before and after treatment | False-negative results possible in the presence of PPIs or with recent use of antibiotics of bismuth preparations, considerable resources and personnel required to perform test |
| Stool antigen test | 96 | 97 | High negative and positive predictive values, useful before and after treatment | Process of stool collection may be distasteful to patient, false-negative results possible in the presence of PPIs or with recent use of antibiotics or bismuth preparations |
| Invasive | | | | |
| Histology | 95 | 99 | Excellent sensitivity and specificity, especially with special and immune stains, provides additional information about gastric mucosa | Expensive (endoscopy and histopathology costs), interobserver variability, accuracy affected by PPI and antibiotics use, requires trained personnel |
| Rapid urease test | 90 | 93 | Rapid results, accurate in patients not using PPIs or antibiotics, no added histopathology cost | Requires endoscopy, less accurate after treatment or in patients using PPIs |
| Culture | 58.1 | 100 | Specificity 100%, allows antibiotics sensitivity testing | Variable sensitivity; requires trained staff and properly equipped facilities, expensive |

PPI, proton pump inhibitor; *H. pylori*, *Helicobacter pylori*.

# BACKGROUND

- In an effort to correlate the histologic features of a lymphoid infiltrate with the likelihood of MALT lymphoma, Wotherspoon et al developed a scoring system from 0 to 5
  - Proven to correlate well with immunoglobulin heavy chain (IGH) gene rearrangement studies for clonality by polymerase chain reaction (PCR)
  - The presence of monoclonality in cases with a Wotherspoon score of $\geqslant 3$ should be regarded as malignant
  - The application of Wotherspoon criteria with IGH studies in a focused pediatric population is limited

**TABLE 1.** A Modified Version of the Wortherspoon[7,8] Criteria Was Used For Scoring the Inflammatory Infiltrate in Gastric Foveolar Biopsies

| Score | Diagnosis | Original Wotherspoon Criteria[7,8] | Modified Wotherspoon Criteria |
|---|---|---|---|
| 0 | Normal | Scattered plasma cells in lamina propria. No lymphoid follicles | Scattered plasma cells in lamina propria. No lymphoid follicles or aggregates |
| 1 | Chronic active gastritis | Small clusters of lymphocytes in lamina propria. No lymphoid follicles. No lymphoepithelial lesions | Small clusters of lymphocytes in lamina propria. No lymphoid follicles or aggregates. Rare lymphocytes in the epithelium (< 3 glands/high-power field). No lymphoepithelial lesions |
| 2 | Chronic active gastritis with florid lymphoid follicle/ aggregate formation | Prominent lymphoid follicles with surrounding mantle zone and plasma cells. No lymphoepithelial lesions | Lymphoid aggregates with or without follicles. Lymphocytes in the epithelium (≥ 3 glands/high-power field). No lymphoepithelial lesions |
| 3 | Suspicious lymphoid infiltrate, probably reactive | Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into the epithelium | Lymphoid follicles or aggregates, with or without germinal centers. Lymphocytes in the epithelium or equivocal lymphoepithelial lesions |
| 4 | Suspicious lymphoid infiltrate, probably lymphoma in the appropriate clinical setting | Lymphoid follicles surrounded by marginal zone cells that infiltrate diffusely in lamina propria and into epithelium in small groups | Lymphoid follicles or aggregates, with or without germinal centers. Lymphoepithelial lesion present (at least 1 gland) but not prominent, and rare |
| 5 | Mucosa-associated lymphoid tissue lymphoma, in the appropriate clinical setting | Presence of dense infiltrate of marginal zone cells in lamina propria with prominent lymphoepithelial lesions | Dense infiltrate of lymphocytes in the lamina propria with prominent lymphoepithelial lesions. Lymphoid follicles or aggregates, with or without germinal centers |

The changes are minor and serve to better clarify the described properties. A lymphoepithelial lesion is defined by a cluster of at least 3 lymphocytes within the glandular epithelium.[9]

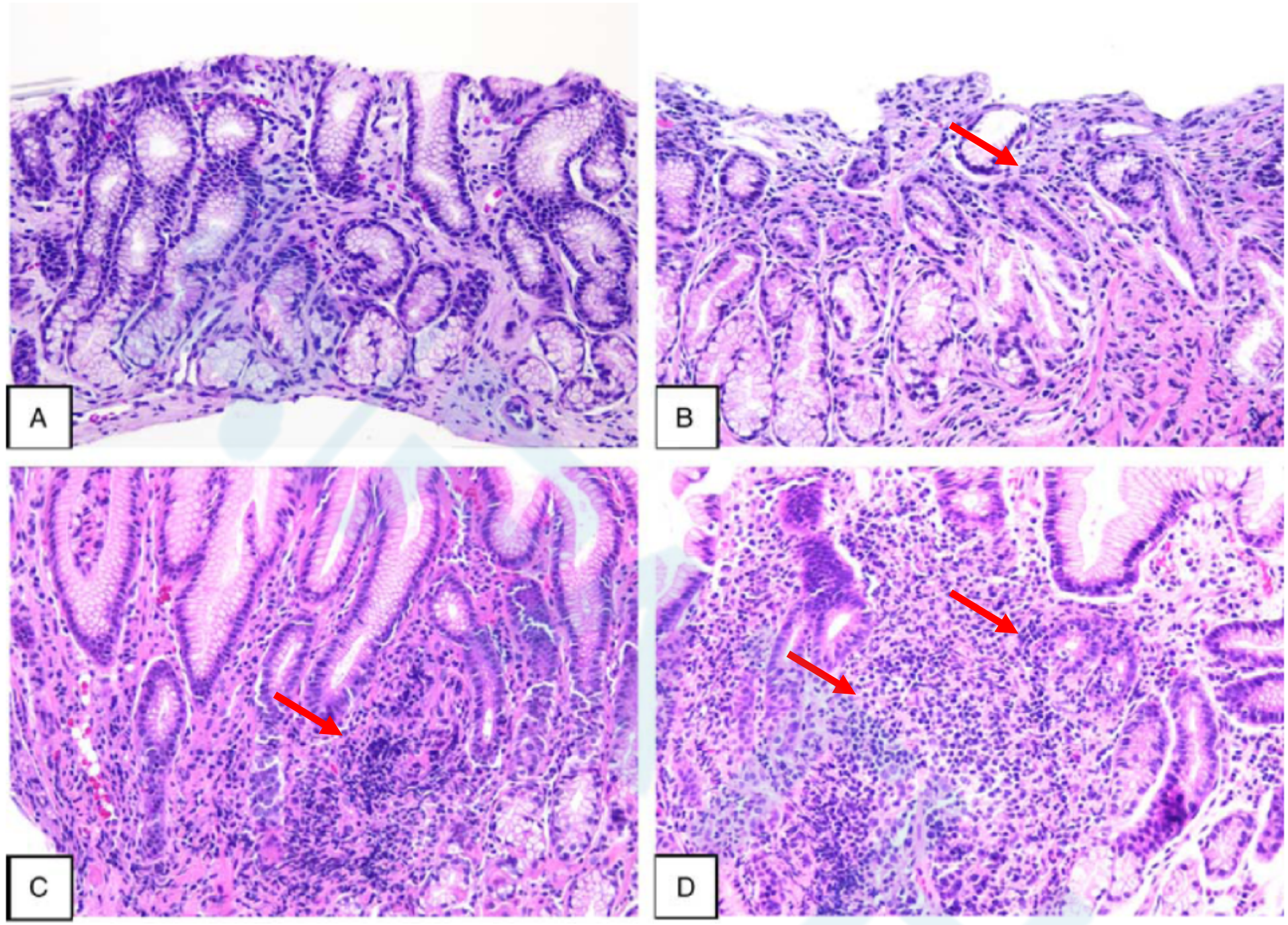
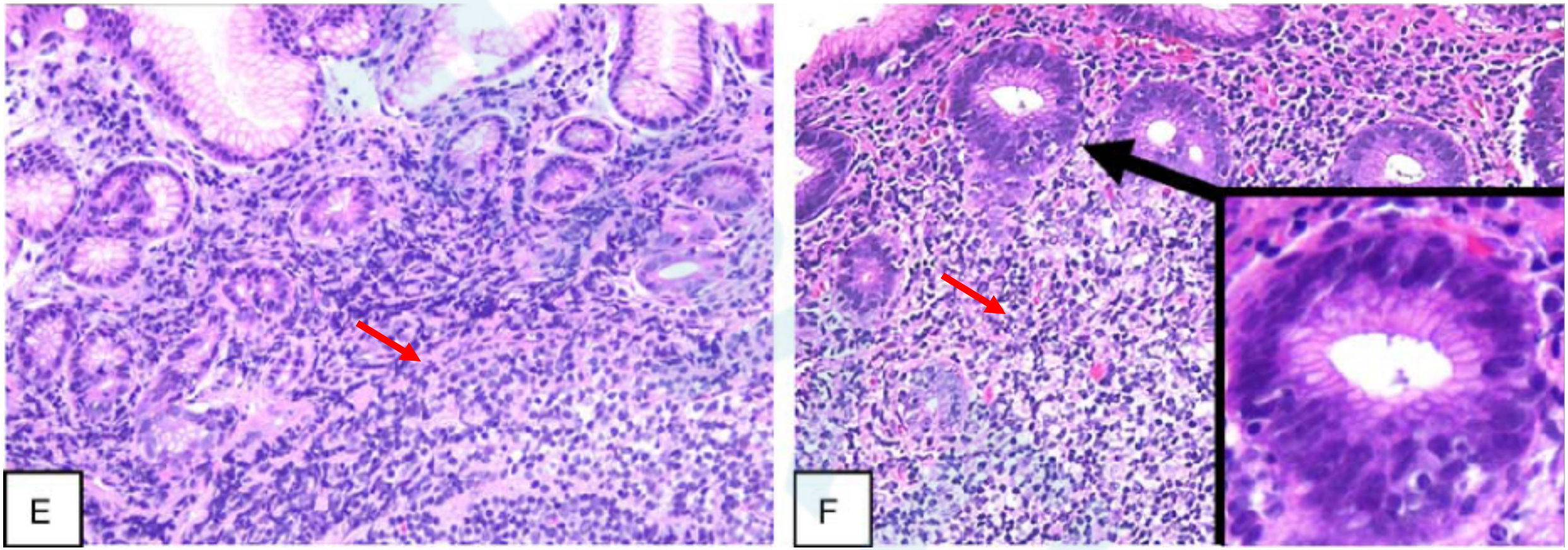



FIGURE 1. Examples of Modified Wotherspoon Scores (MWS) 0 to 5.A, MWS 0 shows predominantly plasma cells. B, MWS 1 shows small clusters of lymphocytes with rare single intraepithelial lymphocytes. C, MWS 2 shows lymphoid follicles with more prominent intraepithelial lymphocytes without clustering. D, MWS 3 shows lymphoid follicles with equivocal lymphoepithelial lesions (clusters of at least 3 lymphocytes).




E, MWS 4 shows lymphoid follicles with rare lymphoepithelial lesions (up to 1 gland/ high-power field). F, MWS 5 shows lymphoid follicles with prominent lymphoepithelial lesions (highlighted in the inset).

# Helicobacter pylori Gastritis in Children and Adults

- In H pylori–infected children
  - The gastric mucosal inflammatory cell infiltrate has been described to be more severe
  - Higher frequency of lymphoid follicles compared with adults
  - Less degrees of acute inflammatory infiltrates in response to H pylori infection
  - Ulcers and intestinal metaplasia were present only in adults
  - The good prognosis of Helicobacter-associated gastritis in this age group
- Increased lymphocytes in a gastric biopsy
  - Concern for MALT lymphoma in adults
  - Less alarm in pediatric cases given the rarity of lymphoma in these patients
- Evaluate the correlation of a high Wotherspoon score with IGH PCR studies, and evaluate the inflammatory infiltrate in pediatric cases in relation to Helicobacter infection

# MATERIALS AND METHODS

- Biopsies within the pediatric (age 18 y or younger) population, diagnosed between 1996 and 2018
  - Helicobacterassociated chronic gastritis (62 cases)
    - Hematoxylin and eosin (H&E), targeted polyclonal antibody IHC , or Giemsa staining
    - For patients with multiple Helicobacter-infected gastric biopsies, the most recent available positive biopsy was chosen
  - Helicobacter-negative chronic gastritis (17 cases)
    - Moderate chronic gastritis with no evidence of Helicobacter by IHC
    - Any patients with positive Helicobacter serology or urea breath test were excluded from the control group

# MATERIALS AND METHODS

- The inflammatory infiltrates were examined in gastric foveolar mucosal fragments on H&E sections
  - The strength of the infiltrate:minimal (⩽ 25% stromal infiltrate), mild (26% to 50%), moderate (51% to 75%), or severe ( ⩾75%)
  - The linear property of the inflammation :patchy,diffuse
  - The depth :superficial or full-thickness
  - Active inflammation the presence of intraepithelial neutrophils
  - Germinal centers were counted within foveolar fragments
  - A modified version of the Wotherspoon criteria score (MWS) was independently scored by 2 pathologists

# MATERIALS AND METHODS

- In situ hybridization (ISH)
  - κ , λ
- Immunohistochemistry
  - CD20 ,CD3, CD5,CD10 ,Epstein-Barr virus–encoded RNA (EBER) ISH
- *IGH* clonality studies
  - Any cases that scored a 3 to 5 on MWS
- T test, χ 2 test or the Fisher exact test

**TABLE 2.** A Summary of the Demographic Features of the Patients and the Characteristics of the Inflammatory Infiltrates

| | n (%) | | |
|---|---|---|---|
| | *Helicobacter*-associated Cases (N = 62) | *Helicobacter*-negative Cases (N = 17) | *P* |
| Average age (y) | 12 | 14 | 0.1 |
| Sex ratio (male: female) | 35:27 | 5:12 | 0.05 |
| Strength of infiltrate | | | 0.7 |
| Minimal (≤25%) | 4 (6) | 0 (0) | |
| Mild (26%-50%) | 9 (15) | 2 (12) | |
| Moderate (51%-75%) | 18 (29) | 7 (41) | |
| Severe (≥75%) | 31 (50) | 8 (47) | |
| Linear property of infiltrate | | | 0.9 |
| Patchy | 30 (48) | 8 (47) | |
| Diffuse | 32 (52) | 9 (53) | |
| Depth of infiltrate | | | 0.2 |
| Superficial | 18 (29) | 8 (47) | |
| Full-thickness | 44 (71) | 9 (53) | |
| Active inflammation | 29 (47) | 2 (12) | 0.01 |
| No. germinal centers | | | 0.1 |
| 0 | 31 (50) | 13 (76) | |
| 1 | 24 (39) | 2 (12) | |
| 2 | 5 (8) | 2 (12) | |
| 3 | 0 (0) | 0 (0) | |
| 4 | 2 (3) | 0 (0) | |
| Modified Wotherspoon Score | | | 0.5 |
| 0 | 3 (5) | 1 (6) | |
| 1 | 16 (26) | 4 (24) | |
| 2 | 14 (23) | 8 (47) | |
| 3 | 7 (11) | 1 (6) | |
| 4 | 16 (26) | 2 (12) | |
| 5 | 6 (10) | 1 (6) | |
| *IGH* results | | | 1.0 |
| Clonal | 7 (27) | 1 (25) | |
| Nonclonal | 19 (73) | 3 (75) | |
| κ:λ ratio | | | 0.7 |
| 1:2 | 4 (6) | 0 (0) | |
| 1:1 | 31 (50) | 10 (59) | |
| 2:1 | 13 (21) | 4 (24) | |
| 3:1 | 3 (5) | 2 (12) | |
| 4:1 | 1 (2) | 0 (0) | |
| Insufficient tissue | 10 (16) | 1 (6) | |

**TABLE 3.** A Comparison of Clonal Cases to Nonclonal Cases Based on *IGH* Polymerase Chain Reaction Results

| | n (%) | | |
|---|---|---|---|
| | **Clonal Cases (N = 8)** | **Nonclonal Cases (N = 22)** | ***P*** |
| Average age (y) | 13 | 12 | 0.3 |
| Sex ratio (male:female) | 3:5 | 15:7 | 0.2 |
| Strength of infiltrate | | | 1.0 |
| Minimal (≤25%) | 0 (0) | 0 (0) | |
| Mild (26%-50%) | 1 (13) | 2 (9) | |
| Moderate (51%-75%) | 2 (25) | 7 (32) | |
| Severe (≥75%) | 5 (63) | 13 (59) | |
| Linear property of infiltrate | | | 0.7 |
| Patchy | 5 (62) | 10 (53) | |
| Diffuse | 3 (38) | 12 (47) | |
| Depth of infiltrate | | | 1.0 |
| Superficial | 1 (13) | 4 (18) | |
| Full-thickness | 7 (88) | 18 (82) | |
| Active inflammation | 2 (25) | 13 (59) | 0.2 |
| No. germinal centers | | | 0.8 |
| 0 | 1 (13) | 5 (23) | |
| 1 | 5 (63) | 13 (59) | |
| 2 | 2 (25) | 3 (14) | |
| 3 | 0 (0) | 0 (0) | |
| 4 | 0 (0) | 1 (5) | |
| Modified Wotherspoon Score | | | 0.5 |
| 3 | 2 (25) | 6 (27) | |
| 4 | 3 (38) | 12 (55) | |
| 5 | 3 (38) | 4 (18) | |

# RESULTS

- Inflammatory infiltrates in both populations were comprised of a mixture of B-cells and T-cells based on CD20 and CD3 staining, respectively
- None of our cases with sufficient tissue on TMA stained for Epstein-Barr virus by EBER ISH
- The average follow-up time was 31months (range: 0 to 216 mo) for Helicobacter-associated patients and 49 months (range: 1 to 81 mo) for Helicobacter-negative patients (P=0.8)
  - None of our patients were documented to have developed a diagnosis of gastric lymphoma or to have died within the short follow-up time span
  - All Helicobacterassociated gastritis patients were prescribed appropriate therapy

# DISCUSSION

- The diagnosis of primary gastric MALT lymphoma can be difficult to distinguish from chronic gastritis
  - Morphologic schemes for the likelihood of each diagnosis have been produced to aid in the separation
  - Assessment of clonality with IGH PCR studies can also aid in the diagnosis of MALT lymphoma, though monoclonality can be present in up to 85% of chronic gastritis cases without overt lymphoma

# DISCUSSION

- None of our patients developed primary gastric lymphoma
  - The average age of diagnosis for MALT lymphoma is over 50 years
  - Predisposing Helicobacter infection is thought to occur in childhood
  - 3 pediatric cases with a MWS of 5 with IGH monoclonality
  - Hesitate to apply the terminology in the pediatric population

# DISCUSSION

- Why MALT lymphoma is rarely diagnosed in the pediatric population
  - In the pediatric population may lie in how well children do despite Helicobacter infection given the seemingly long prophase of MALT lymphoma
  - This population is already receiving the proper treatment necessitated

- The progression from nonhistologic lymphoma with monoclonality to histologic lymphoma with monoclonality could occur despite anti-Helicobacter therapy
- These patients may be at higher risk for later development of primary gastric MALT lymphoma and may have benefited from closer surveillance

# DISCUSSION

- The limitations of this study
  - Small sample size and only 1 institution's data
  - The lack of Helicobacter staining on IHC in our Helicobacter-negative cases can be secondary to sampling error
  - The possibility of false positives on IGH testing as a result of a low number of lymphocytes analyzed
  - Lacks genetic analysis for the common MALT lymphoma-associated translocations
- Future studies evaluating this could be considered

# CONCLUSION

- Although cases could be considered MALT lymphoma in an adult patient based on prominent lymphoepithelial lesions and IGH monoclonality, caution is advised when diagnosing lymphoma in the pediatric population given the good prognosis of Helicobacter-associated gastritis in this age group

# THANK YOU