Oral ulceration is quite complicated and diverse. In addition to some cases that can be attributed to local stimulus, including mechanical (sharp edges of residual root or crown, etc.), physical (thermal burns, etc.), or chemical (strong acid or alkali, etc.) factors, most oral ulcers occur due to the combination of both local and systemic causes. The diagnosis and treatment of oral ulcers in various types are tasks of oral specialists. Aside from a large number of common cases, more rare cases of oral ulcers have been recorded. Often, it is difficult to provide immediate and definitive diagnosis for the latter form of cases.

Oral ulceration is characterized by the persistent defect or destruction in the integrity of the oral epithelium, accompanied by variable loss of the underlying connective tissue, resulting in a crateriform appearance. From the aspects of etiology, causes of oral ulcers are related to traumatic, infectious, allergic factors, and may be associated with skin disease, autoimmune disease, tumor, inflammatory bowel disease, and so on. However, the exact cause is unknown in some cases. For example, recurrent aphthous ulcers (RAU) may be caused by disturbed immune response, genetic predisposition, nutrient deficiency, oral trauma, anxiety or stress. None of these factors has been confirmed. The complexity of oral ulcerations poses considerable diagnostic and therapeutic challenges to oral specialists. The expert consensus was conducted to summarize the diagnostic work-up for difficult and complicated oral ulcers, based on factors such as detailed clinical medical history inquiry, histopathological examination, and ulceration-related systemic diseases screening. Not only can it provide a standardized procedure of oral ulceration, but also it can improve the diagnostic efficiency, in order to avoid misdiagnosis and missed diagnosis.

The diversity and complexity of oral ulcerations pose considerable diagnostic challenges to oral specialists. To improve the diagnostic accuracy and timeliness, especially to improve outcomes and survival of those patients with oral ulceration caused by systemic precursor, and to reduce unnecessary financial burden, National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University takes the lead to summarize the diagnostic work-up for difficult and complicated oral ulcerations by various types.
oral ulcers. The 23 experts in oral medicine from 21 different institutions all over China were invited to discuss and conduct this expert consensus (Fig. 2). This process is decomposed into three parts and will be introduced respectively.

PART 1
For oral ulceration that cannot be diagnosed after collection of clinical medical history and oral examination, especially those with the course over 2 weeks, or cases which do not respond to 1–2 weeks of treatments, a biopsy should be considered. Note that blood test is necessary before the biopsy, aiming to exclude contraindications. More importantly, blood test can also provide clues of further clinical examination and diagnosis.

Blood tests include full blood count, coagulation, fasting blood glucose level, HIV antibody, and syphilis serology examination. Full blood count can find the trail of blood system diseases. If anemia or leukemia is suspected, the diagnosis should be made by more tests such as blood iron, folate, vitamin B12, bone marrow biopsy, immunotyping, etc (Fig. 3). Blood coagulation and fasting blood glucose are designed to exclude biopsy contraindications. Because hyperglycemia is an important predisposing factor of invasive fungal infection, oral ulcers caused by a fungal infection should be considered in patients with high blood glucose. Detection of HIV antibody and syphilis serology examination help to rule out oral ulceration associated with HIV and syphilis infection. In addition, evaluation for serum specific antibodies, such as Dsg1, Dsg3, BP180, and BP230 before biopsy, is crucial in patients with suspected bullous diseases.

PART 2
If the diagnostic clues cannot be found by blood test before a biopsy, and there is no contraindication, evaluation of the oral ulceration will enter the Part 2—a biopsy. The following issues need to be considered before and during the biopsy. First, if ulcers involve multiple sites with different morphological characteristics, multiple biopsies should be considered. Second, if bullous diseases are suspected clinically, direct immunofluorescence (DIF) accompanied with HE staining are required. Due to the different submission and specimen handling methods, adequate tissue is needed for both procedures. For HE, the tissue (one from lesional and perilesional site with intact epithelium) in 10% formalin is processed using routine techniques. But DIF specimen is obtained from adjacent normal tissue, Michel's buffer or normal saline is used as transport medium. In addition, the specimen requires some normal-appearing tissues and adequate depth, especially the ulcerative lesions clinically suspected to be the lymphoma.

HE staining of biopsy specimen may suggest the diagnosis in most cases, mainly including cancerous ulcer (carcinoma in situ or oral squamous cell carcinoma), bullous diseases, hematopoietic and lymphoid neoplasm, granulomatous inflammation, acidophilic ulcers, etc. Final diagnosis still need subsequent examinations. If suspected bullous diseases are detected by HE staining, DIF, indirect immunofluorescence, and enzyme-linked immunosorbent assay with recombinant autoantigen should be combined with. If the lesion is suggestive of hematopoietic and lymphoid neoplasm, further examinations such as immunohistochemical assay, T-cell receptors gene rearrangement, bone marrow aspiration, and immunophenotyping are needed (Fig. 4). Moreover, if granulomatous inflammation is indicated, tuberculosis can be eliminated by acid-fast staining and TB DNA detected by fluorescence quantitative PCR; invasive fungal infection can be excluded by periodic acid-Schiff stain and gomori's methenamine silver nitrate stain. Non-infectious granuloma caused by Crohn's disease and granulomatous vasculitis (Wegener's granulomatosis) may also be considered. The final diagnosis of Crohn's disease depends on colonoscopy, gastrointestinal biopsy, and gastrointestinal CT scan. And diagnosis of granulomatous vasculitis is made by combination of nasal lesion, chest CT, urine routine, and antineutrophil cytoplasmic antibodies.

PART 3
Sometimes patients are not eligible to have a biopsy due to contraindications or poor general conditions. Even if the oral biopsy is carried out, a definitive diagnosis is often difficult.
Complicated oral ulcers cannot be diagnosed after medical history collection and oral examination

Examinations before biopsy

- Full blood count
- Rule out the diagnosis of blood system disease such as anemia and leukemia
- Blood coagulation
- Rule out surgical contraindication
- Fasting blood glucose
- Rule out diabetes (susceptible factor of invasive fungal infection)
- HIV antibody
- Rule out HIV and TP infections
- Syphilis serology

Biopsy (HE staining, consider DIF or IHC)

Part 1

Full blood count
Blood coagulation
Fasting blood glucose
HIV antibody
Syphilis serology

Part 2

- Positive findings
  - Bullous diseases
  - Eosinophilic ulcer
  - Lymphoid hematopoietic malignancies
  - Granulomatous inflammation
  - Others Pyostomatitis vegetans, etc.

- Negative findings
  - Lymphoma (NK/T cell lymphoma, etc.)
  - Leukemia
  - Myeloid sarcoma
  - Langerhans cell histiocytosis
  - Others
  - Tuberculosis
  - Invasive fungal infection
  - Syphilis
  - Granulomatous angiitis
  - Crohn’s disease
  - Sarcoidosis
  - Others

Part 3

- Examinations
  - Tuberculosis
  - Diabetic
  - Intestinal diseases
  - History of radiotherapy, chemotherapy, medication, drug abuse
  - Specific antibiotics of bullous diseases
  - Anti-neutrophil cytoplasmic antibodies
  - Granulomatosis with polyangiitis
  - HLA-B27
  - Reiter syndrome
  - Metagenomic sequencing, microbial cultures, etc.
  - Infectious disease caused by rare pathogenic microorganism
  - Whole-exome sequencing, etc.
  - Genetic diseases such as dyskeratosis congenita

Note: According to the actual conditions, inspection items could be synchronous, diagnostic treatment can be made ahead of time, as appropriate

If no abnormal findings
- Try diagnostic treatment; MDT consultation; A second biopsy if necessary

Fig. 2 Diagnostic work-up for difficult and complicated oral ulcers

Fig. 3 A 22-year-old man with oral ulcers for 3 days. Widespread necrotic ulcers on the left maxillary gingiva, extending to the hard palate, with smooth and thick yellowish-white pseudomembrane. Full blood count showed the percentage of neutrophilic segmented granulocytes decreased significantly (2.0%). Then the bone marrow biopsy and immunotyping revealed acute monocytic leukemia, type M5

to obtain. For instance, the pathological findings that “inflammatory ulcer, infiltration of lymphocyte was found in the submucosa” is a common challenge in clinical practice with no specific significance. In this case, we consider taking paraffin-embedded specimens to the superior pathologists for consultation (Fig. 5).

Evaluation of oral ulceration will get into Part 3 if patients with inoperable conditions or the diagnosis remain unestablished after consultation. That is, we step back and perform further screening of ulceration-related systemic diseases.

Fig. 4 A 25-year-old woman with ulcer for 2 months. Serious erosion and necrosis on the upper labial mucosa, extending to anterior maxillary gingiva covered by yellowish-white pseudomembrane. HE staining and immunohistochemical studies of the biopsy supported the diagnosis of nasal-type extranodal NK/T-cell lymphoma.
Detailed medical history should be collected again, focusing on common illnesses such as tuberculosis, diabetes, and intestinal diseases (Fig. 6). Moreover, the history of radiotherapy, chemotherapy, medication, drug abuse should also be reconfirmed. Besides, further examinations as appropriate should be considered.

For oral ulcers located on or near the hard tissue, maxillofacial CT examination would find out whether there is bone destruction or not. Otolaryngology consultation, nasal spiral CT, and nasopharyngoscopy will be performed if necessary. Chest CT examination helps to rule out tuberculosis, invasive fungal infection, and paraneoplastic syndrome. Tuberculin skin test (PPD) and interferon gamma release assay (TB-IGRA) are useful to exclude tuberculosis. Invasive fungal infection can be detected by 1-β-D-glucan chromogenic assay and galactomannan (GM) assay. 1-β-D-glucan is a cell wall polysaccharide component, and GM is another component of the fungal cell wall, which is detected for early diagnosis of fungi, particularly the most common pathogens such as Aspergillus and Candida, but also has clinical value for the diagnosis of invasive fungal infection (Fig. 7). Oral glucose tolerance test, glycosylated hemoglobin, and urine sugar are the screening methods of diabetes, especially latent or early diabetes. Specific antibodies such as Dsg1, Dsg3, BP180, and BP230 can provide clues to bullous diseases. Other methods include metagenomic sequencing, microorganism cultivation of ulcerative tissues, immune function measurement, and so on.

If there is no abnormality after the above medical history collection and additional examinations, diagnostic treatment with low-dose and short-term oral glucocorticoids, a second biopsy, or the multi-disciplinary team consultation may be performed if necessary. Screening for genetic diseases by whole-exome sequencing should also be concerned. Note that inspection items in this diagnosis process may be synchronous according to the actual conditions. Examinations of Part 3 can be made before the biopsy, and a diagnostic treatment could also be performed in advance.

There are numerous causes of oral ulcerations. This diagnosis process only involves common causes without covering all factors. With the development of detection technology and clinical thinking ability, we will further perfect this diagnosis process to provide useful reference and guidance for oral specialists.

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ADDITIONAL INFORMATION
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