Current Diagnostic Methods of Gut Microbiota

Subjects: Transplantation

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The human gut microbiota include over 10 trillion microorganisms, such as bacteria, fungi, viruses, archaea, and protozoa. In the past, the gut microbiome was analyzed using isolation and culture.

dysbiosis gut microbiota orthotopic heart transplantation

1. Introduction

Cardiovascular diseases are still the leading cause of reduced quality of life, morbidity, and mortality in developed societies [1][2]. End-stage heart failure (HF) is a severe, final stage of many cardiovascular diseases. Epidemiological data show that the incidence of heart failure varies between 1% and 2% and increases to 10% in people over 70 years of age [3][4][5]. The prognosis after the diagnosis of heart failure is poor: five-year survival is less than 50%. Although significant progress has been made in the treatment of HF, there are still two vital treatment options for this condition: left ventricular assist devices (LVAD) and heart transplantation (HT) [6][7][8]. There is much information in the literature on the importance of the gut microbiome in cardiovascular diseases, including in patients treated with LVAD and after HT [9][10][11].

The human gut microflora includes over 10 trillion microorganisms, such as bacteria, fungi, viruses, archaea, and protozoa. The microflora in a healthy person consists mainly of the following groups of bacteria: Bacteroides, Actinobacteria, Proteobacteria, Firmicutes, Verrucomicrobiota, Cyanobacteria, Fusobacteria, Spirochaetes, and Vadin BE97, with Bacteroides and Firmicutes accounting for 90% of the total bacterial population. The phylum Firmicutes consists of 95% Clostridium, while the majority of Bacteroides are Prevotella and Bacteroides. This composition of microorganisms is constantly modified [12]. Reduced microbial diversity comprising the depletion of potentially beneficial saprophytic microbiota (Bacteroides and Firmicutes) and the overgrowth of pathogenic bacteria is called dysbiosis [13].

2. Current Diagnostic Methods of Gut Microbiota

1. Samples from feces

This is a non-invasive, natural, inexpensive test that can be repeated many times. However, it should be noted that there are significant differences between the microflora found in feces and that found in the intestinal mucosa. Recent studies have shown that fecal microflora, compared to that associated with mucous membranes, is

composed of two distinct microbial niches [14][15]. In addition, the fecal microflora is not evenly distributed in the feces and has its own biostructure. Different bacteria are found in the small intestine, and others in the final section of the digestive tract. The need for the proper storage of fecal samples should also be considered [16].

2. Samples from endoscopy:

- Biopsy

  Few studies have been conducted on endoscopy samples. Much has been said about the disadvantages of this method. Among other things, it is invasive, expensive, time consuming, and unpleasant for patients.

  Before the endoscopy, the patient should be adequately prepared for the examination. Laxatives, such as polyethylene glycol, should be given, which significantly impacts the gut microflora. In addition, using endoscopy, it is unavailable to reach the final section of the small intestine, and the material is only collected from a small area of the intestine, which can give selective results. Moreover, the collected material may be contaminated with the fluid in the sampling device. To minimize the risk of sample contamination, a unique biopsy device (Brisbane Aseptic Biopsy Device (BABD)) has been developed, which consists of sterile forceps covered with a sheath and sealed with a plug at the ends. The advantage is a controlled sampling site and the ability to obtain an accurate description of the tissue-associated microflora [17][18].

- Luminal brushing

  The protected specimen brush (PSB) is a disposable, sterile brush that is housed in a special cover with a cap, which is sealed when inserted through the colonoscopic canal. Compared to a biopsy, brushing the intestinal mucosa reduces the risk of bleeding or infection and is less invasive. Additionally, luminal brushing contains a relatively high ratio of bacterial DNA to host DNA. The advantage is, as in the case of a biopsy, a controlled sampling site and the possibility of obtaining an accurate description of the microflora associated with the tissue. Samples are also taken during the endoscopic examination; therefore, this method has the same disadvantages as a biopsy—the intestine must be adequately prepared, which results in a change in the diversity of the microflora. It is also an invasive, time-consuming, and expensive method [19][20].

- Laser capture microdissection (LCM)

  The source of the sample is also a biopsy and therefore suffers from the same disadvantages as the two previously mentioned methods. Then, the collected material is properly and precisely prepared—this limits the use of the method on a large scale. Then, with the help of a laser, the microflora is carefully analyzed. The advantage is an accurate representation of the interaction between the host and the microbiome, as well as a controlled sampling site [19][21].

3. Samples from aspirated intestinal fluid

This method involves suctioning out the intestinal fluid. At present, samples of aspirated intestinal fluid are collected by endoscopic aspiration. This method has the same disadvantages that have been mentioned with endoscopy—it is unavailable to reach the final segment of the small intestine, and the material is only collected from a small area of the intestine, which can give selective results. In addition, the collected material may be contaminated with fluid in the sampling device. It is also a time-consuming procedure, causing patient discomfort. The advantage is the ability to obtain a precise description of the luminal microflora and a controlled sampling site [22].

4. Samples from surgery

Unlike other methods, this method enables the collection of material from the final section of the small intestine. The material during the procedure is collected by needle aspiration or biopsy. It is said that samples taken using this method best reflect the composition of the microflora and are not contaminated. The exact place where the sample was taken are also controlled. On the other hand, the proper preparation of the patient for the procedure, in the form of administering, for example, laxatives or antibiotics, may significantly disturb the composition of the gut microflora. It is a very invasive method [22].

5. Ingestible sampling devices

This is a non-invasive method, consisting of the patient swallowing a special capsule, which aspirates the food in the right place in the intestine. The aspirated fluid can be collected after the capsule is emptied from the intestine. Thanks to the lack of the necessity to prepare the intestine, it is a more accurate and precise method—it does not change the composition of the gut microflora. Thanks to this method, an accurate description of the luminal microflora can be obtained. There is also less risk of contamination of the collected sample. It is a relatively expensive and technically complicated method [23].

6. In vivo model (patients who underwent ileostomy)

This method is dedicated to patients who have had an ileostomy. This procedure significantly changes the anatomical structure of the intestine, which can have a significant impact on the composition of the gut microflora. It is a relatively inexpensive, non-invasive method. Reproducible sampling (sufficient biomass for analysis) can be performed and the risk of sample contamination can be minimized [24].

7. Biology-related instruments

The method used to identify microorganisms is the FISH method (fluorescence in situ hybridization), which, with the usage of a fluorescence microscope, allows to accurately identify the spatial organization of the microbiome. Improved histological preparations allow, in addition to identifying microbial diversity, the ability to determine the host–microbiota correlation. Due to ethical problems, difficulties in sampling (the probe used to collect the material must be designed in advance), and high individual variability in the microbial composition, the application of this method in situ is limited [25].

At the moment, there is no single, ideal method for collecting samples of the intestinal microbiome. Research on this is still ongoing.

The current gut microbiota diagnostic methods and their advantages and disadvantages are summarized in **Table 1**.

Table 1. Current diagnostic methods for gut microbiota.

Diagnostics Methods	Advantages	Disadvantages
Samples from feces	Non-invasive, natural, inexpensive, suitable biomass for analysis, repeatable, convenient	Uneven distribution of bacteria in the feces may lead to erroneous results
Samples from endoscopy 1. Biopsy	Controlled sampling site, ability to obtain an accurate description of the tissue-associated microflora	Invasive, expensive, time consuming, and unpleasant for patients, contamination of the sample may occur, need to prepare the patient accordingly, insufficient biomass sample
Samples from endoscopy 2. Luminal brushing	Controlled sampling site, ability to obtain an accurate description of the tissue-associated microflora	Invasive (less invasive than a biopsy), expensive, time consuming, and unpleasant for patients, contamination of the sample may occur, need to prepare the patient accordingly
Samples from endoscopy 3. Laser capture microdissection	Controlled sampling site, ability to obtain an accurate description of the tissue-associated microflora and host–microbe interactions	Invasive, expensive, time consuming, and unpleasant for patients, contamination of the sample may occur, need to prepare the patient accordingly, insufficient biomass sample, is not appropriate for healthy control
Samples from aspirated intestinal fluid (catheter aspiration)	Controlled sampling site, ability to obtain an accurate description of the tissue-associated microflora	Invasive, time consuming, and unpleasant for patients, contamination of the sample may occur, need to prepare the patient accordingly
Samples from surgery	Controlled sampling site, ability to obtain an accurate description of the tissue-associated microflora, no contamination	Possible influence of preoperative preparations on the microflora, very invasive, is not appropriate for healthy control
Ingestible sampling devices (intelligent capsule)	Non-invasive, ability to obtain an accurate description of the tissue-associated microflora, no need to properly prepare the patient, sample contamination does not occur	Technically difficult and expensive
In vivo model (patients underwent	Non-invasive, inexpensive, sample contamination does not occur,	Abnormal intestinal anatomy, is not appropriate for healthy control

Diagnostics Methods	Advantages	Disadvantages
ileostomy)	repeatable and convenient sampling, sufficient biomass for analysis	
Biology-related instruments (FISH)	Ability to obtain an accurate description of the tissue-associated microflora and host–microbe interactions	Not suited to a complex microbiome, the sampling device must be designed in advance

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