Blood metagenome in health and psoriasis.

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Abstract

Survey and analytical assessment of the results of fundamental works on studying metagenomes of blood and skin is carried out. All works on determining bacterial DNA concentration in whole blood of healthy people are reviewed.

Detailed comparison of characteristics of 16S-test and WMS-test is carried out and published in Supplement S1.

One of main goals of this review is to identify the drawbacks and mistakes which the studied works contain, particularly to emphasize the crucial importance of determining total concentration of bacterial DNA for comparing patients’ metagenomes with those of healthy people as well as for comparing patients’ metagenomes with each other.

Controlling the level and composition of contamination is equally important. The absence of high-quality contamination control at each step (or at certain steps) of the research significantly reduces the reliability of achieved results.

The given review is the first attempt to analyze and systematize the results of blood metagenome studies, whose number has increased considerably in the last few years. The review has been carried out as part of preparation for implementing a project on complex studying metagenomes of whole blood and skin biopsies at psoriatic patients.

Keywords

Bacterial DNA, bacterial products, bioinformatics, intestinal permeability, lipopolysaccharide, metagenome, microbiome, netosis, neutrophils, non-host DNA, peptidoglycan, phagocytes, psoriasis, psoriatic disease, sequencing.

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1. Introduction

Epidermis self-renewal is a regular process. New cells are born in basal layer. They mature, vary, migrate outside and form external horny layer. Then they die away and exfoliate. Standard duration of epidermis cell life (renewal period) for areas of skin with average thickness is 20-25 days. Psoriasis accelerates self-renewal. Cells live 4-10 days. Cells migrating outside have no time to differentiate and they aren't quite functional. Psoriatic plaques have red shade. They are tender, they are covered by white flakes due to intensive lost of cells and they are much thicker.

Psoriasis isn't contagious. There are various types of psoriasis: vulgaris or plaque (L40.0), flexural or inverse (L40.83-4), erythrodermic (L40.85), pustular (L40.1-3, L40.82), guttate (L40.4). Codes of diseases are given according to ICD-10. Chronic plaque psoriasis is the most frequent type (more than 80% of total number of cases). Up to 15% of psoriatics also suffer from psoriatic arthritis (L40.5).

Psoriasis strikes about 2% of population (~150 million people). New diagnosis of psoriasis gets ~5 million people every year. Disease appears after birth or in extreme old age. Psoriasis is a chronic disease so there are periods of aggravation and remission. Sometimes there is no cause for period change and sometimes aggravation can be decreased as a result of treatment. Serious psoriasis can result in disability. Psoriasis course is similar in men and women. Afro-Americans, Indians, Chineses and Japaneses suffer from psoriasis less frequently and Eskimos don't suffer from psoriasis at all.

Psoriasis is registered in "Online Mendelian Inheritance in Man" at number OMIM*177900. Psoriasis is disease with hereditary predisposition: concordance of uniovular twins is 70%. If one parent suffers from psoriasis children are diagnosed the disease in 15-25% of cases; if both parents suffer from psoriasis children are diagnosed the disease in more than 40-60% of cases. The interrelation of allele HLA-Cw*0602 (chromosome 6p21) and psoriasis of the first type which is characterized by early beginning is proved. This allele is found in more than 60% of PP (not more than 15% of HP). Locuses of other chromosomes have weaker interrelations. Psoriasis can't begin only in presence of genetical deflections. External exposure is necessary for beginning and maintenance of psoriasis. Infections, skin traumas, stresses, reaction to medications, climatic changes and other causes can provoke onset of psoriasis or its aggravation.

Psoriasis is frequently accompanied by general diseases, including metabolic syndrome, diabetes mellitus of the II type, coronary heart disease, arterial hypertension, pathology of hepatobiliary system.

2. Metagenomic sequencing

The blood metagenome has been frequently identified for its various fractions, both for patients with various diseases, and for healthy persons. It has been primarily identified in plasma or serum (the majority of works), but also in buffy coat, platelets and erythrocytes, whole blood or in neutrophils (Païssé 2016, Li 2018, Puri 2018, Qian 2018) (Table 1).

bacDNA (bacterial DNA) concentration in plasma is more than 3 orders lower than in buffy coat: 1.5*10⁴ (16S copies)/ml against 4.2*10⁷ (16S copies)/ml (Païssé 2016). It is due to this that in many cases bacDNA in plasma was not found at all or, even if it was found, it was only in some patients. The fact is that all bacterial products (as well as any non-host ones, including nhDNA), which get into blood, are constantly utilized.

For the purposes of discussion their utilization can be subdivided into two main ways: phagocyte-dependent (binding, endocytosis) and phagocyte-independent. Phagocyte-independent utilization is assured by degrading enzymes, proteins and antibodies, which connect bioproducts in complexes subsequently brought out of blood flow through elimination organs (primarily kidneys and liver). Utilization of non-host bioproducts can be considered completely phagocyte-independent if their degradation occurs without phagocyte participation. Utilization of non-host bioproducts is phagocyte-dependent if they are endocytosed (binded) by blood phagocytes and at the time of endocytosis (binding) can still be recognized as non-host. Inside phagocytes utilization process of non-host bioproducts definitely continues, though for some time all of them can still be recognized (Fig.1).

This very process occurs at destruction of blood phagocytes and subsequent identification of all DNA – a certain part of it proves to be nhDNA. nhDNA concentration in blood phagocytes appears to be considerably higher than in plasma. I.e. at each timepoint most of nhDNA present in blood is in blood phagocytes (Païssé 2016).

Studies of the presence of bacDNA in PP and HP blood began as far back as in the last century. Let us pass over to a detailed review of the results.

In (Wang 1999) blood plasma of patients with psoriatic arthritis was investigated; in 9 PP out of 19, bacDNA of Streptococcus pyogenes, Str.agalactiae and Str.pneumoniae were found. Their presence was determined by PCR method with specific primers for these species. Metagenome was not identified.

In (Okubo 2002) on several primers for 16S rRNA the presence of bacDNA in blood monocytes was identified in 15 PP and 12 HP (bacDNA was found in all PP and HP). It was discovered that in PP it is...
considerably higher than in HP. Concentration of bacDNA amounted to ~ 3.1 (16S copies)/monocyte for HP and ~ 5.8 (16S copies)/monocyte for PP on average (Okubo 2002, fig.2).

Similar comparison was made on several primers for 18S rRNA (average excess for PP compared to HP is 1.5 times). The authors assumed that the main source of bacDNA was intestine microbiome. Metagenome was not identified.

In (Munz 2010) by 16S-test the presence of bacDNA in plasma of peripheral blood of 20 PP and 12 HP was detected; it was found in all PP and in no HP. In 17 PP the discovered bacDNA was identified as belonging to bacteria from genera Streptococcus or Staphylococcus (Munz 2010, tab.1). Their presence was determined by PCR method with specific primers for these genera. Metagenome was not identified.

In a short article (Ramírez-Boscá 2015) it is reported that by 16S-test bacDNA in blood serum is found only in 16 PP out of 54 and in none of 27 HP. Another study of the same material has been published recently, which also contains results of 16S-test of fecal metagenome of 52 PP (Codoner 2018).

The fact that bacDNA was not found in blood plasma in HP, and frequently in most PP (whereas researches of whole blood were not conducted), made it difficult to make any assertions about the role of its presence in blood in PD pathogenesis. But in recent years there has been considerable improvement of research techniques: hDNA elimination methods, enhancing of accuracy and reliability of results in detecting small quantities and, most importantly, phenomenal depreciation of whole metagenomic sequencing (WMS-tests). For detailed comparison of 16S-tests and WMS-tests see Supplement S1.

Let us outline the main results of these works.

In (James 2011) concentration of bacDNA and hDNA in blood and saliva of donors was studied, their percentage in all DNA isolated from whole blood and saliva respectively was determined. Metagenome was not identified.

In (Amar 2013, Amar 2011) the results of long-term research involving more than 5000 people were summarized. The main objective of this research was identifying the reasons and conditions provoking diabetes. The research was initiated by D.E.S.I.R. Study Group. Among numerous examinations conducted within 9 years (at 3-year intervals) there was quantitative 16S-test of blood leukocytes. The procedure of receiving bacDNA with the maximum concentration in sample was elaborated. In (Amar 2011) we can find comparison of results for patients with diabetes and without one, in (Amar 2013) – for patients with cardiovascular diseases and without them. In (Amar 2011) for a small part of patients with diabetes (n=14) and control group without one (n=28) qualitative 16S-tests were carried out, which made it possible to determine bacterial representation to within genus. In (Amar 2013) metagenome was not identified.

In (Dinakaran 2014) 80 patients with cardiovascular diseases and 40 HP were examined. Blood plasma was used as biomaterial. For all patients 16S-test was used, and for 3 patients 3 HP WMS-test was additionally applied.

In (Sato 2014) 50 patients with type 2 diabetes and 50 HP were examined. By 16S RT-PCR test fecal microbiome and blood plasma microbiome was studied with a limited set of primers (for 21 species, genus or phylum of bacteria). 16S RT-PCR test of blood plasma was qualitative (yes/no), the presence of bacDNA in blood plasma was registered in 14 patients and in 2 HP. Metagenome was not identified. The search of correlations between bacterial genera found in feces and in blood was not carried out.

In (Long 2016) 78 post-surgery patients and 10 HP were examined. WMS-test for blood plasma was carried out, accompanied by bacterial culture (Long 2016, tab.1). The presence of nhDNA was determined (bacteria to within species, fungi and viruses), mapping was carried out on reference catalogues of NCBI Genome. The majority of reads was mapped on human genome (95.6% on average), less than 1% of the rest was mapped on genomes of particular bacteria, fungi or viruses.

In (Païssé 2016) 16S-test of whole blood as well as its fractions was carried out on 30 HP (donors) in order to detect bacDNA. The method elaborated and tested by the authors earlier was applied (Lluch 2015). It turned out that bacDNA is found in all HP and also that its greater part is found in buffy coat, i.e. in the fraction of leukocytes and platelets (93.7%), and the smaller part is connected with the fraction of erythrocytes (6.2%) and blood plasma (0.03%). This is mainly bacDNA of Gram(-) bacteria of phylums Proteobacteria (87%) and Bacteroidetes (classes Sphingobacteria, Bacteroidia and Flavobacteria) (2.5%), but also mainly Gram+ bacteria of phylums Actinobacteria (6.7%), Firmicutes (class Bacilli) (3%).

This research did not aim at establishing the sources of bacDNA origin in blood. Therefore it remained unclear
- if living or degraded bacteria which appeared in blood were source of the discovered bacDNA;
- how bacDNA was connected with leukocytes, platelets and erythrocytes;
- where bacteria and/or bacterial products containing bacDNA got into blood from.
Note that bacDNA concentration found in blood plasma – \(1.4 \times 10^5\) (16S copies)/ml on average – is of the same order with bacDNA concentration found in the control test of one of the reagents – \(1.5 \times 10^5\) (16S copies)/ml.

The results for whole blood have sufficient reliability as bacDNA concentration amounted from \(1.8 \times 10^7\) to \(7.6 \times 10^7\) (16S copies)/ml. On average \(4.2 \times 10^7\) (16S copies)/ml. Direct correlation between bacDNA concentration and leukocyte concentration in blood was demonstrated.

Expressed in terms of leukocytes bacDNA concentration amounted to \(~5.7\) (16S copies)/leukocyte (Païssé 2016, suppl. fig.2a). And as phagocytes are responsible for utilizing non-host bioproducts in blood, it corresponds to \(~8.5\) (16S copies)/phagocyte. It exceeds the concentration in ~3.1 (16S copies)/monocyte for HP (Okubo 2002), if only because in this study postprandial blood was investigated.

The authors assume that the main source of bacDNA income into blood is intestine microbiome. And bacDNA distribution for phylums similar to the one in mucous biopsies of small intestine taken from Treitz ligament in HP is valid (Li 2015). Higher total bacDNA concentration in blood (compared to other studies) is connected with the fact that HP were donors and took food and drink before blood donation (as is often recommended to donors, and also according to the conditions of this research).

It is known that food intake leads to rapid growth of small intestine microbiome (more than by 50 times with celiac disease and with irritable bowel syndrome). This, in its turn, causes temporary growth (presumably the same) of bacterial products income in systemic blood flow (Ciampolini 1996). First it happens in connection with microbiome growth (waste products), and then (in the process of himus move on GIT) because of microbiome reduction (dying off products). In dying off products peptidoglycan PG, lipopolysaccharide LPS and other PAMP constitute a considerable proportion.

In (Lelouvier 2016) the (Luch 2015) method was applied to 16S-tests of buffy coat (patients in Spain) and whole blood (patients in Italy). Each group of patients was divided into two parts (with or without fibrosis). Venipuncture was carried out after 12-hour abstinence from food. A considerably smaller (in comparison with Païssé 2016) bacDNA concentration – about \(2.4 \times 10^5\) (16S copies)/ml for groups without fibrosis and 1.5-2 times more for groups with fibrosis – was found.

In (Grumaz 2016) the following three groups were examined: patients with sepsis (n=60), patients after abdominal surgery (n=30) and HP (n=30). DNA which is found free in blood plasma (cell-free DNA) was studied. Its concentration was determined by means of Qubit dsDNA HS Assay Kit (LifeTechnologies). Then WMS-test was carried out, and reads belonging to hDNA were analytically excluded (96-98% on average).

The other reads were mapped to within species with the use of NCBI RefSeq (reference genomic DB) (tab.1, Grumaz 2016). As a result, averagely from 2.3% to 4.2% of not excluded reads were mapped, for 12 HP – averagely 3.5% of not excluded reads, which corresponds to averagely 0.064% of all reads. Information of nhDNA representation was eventually obtained.

In (Gyarmati 2016) only patients with suspected sepsis (n=9) were examined. nhDNA of non-host cells found in blood was studied. Before sequencing, consecutive enrichment of biomaterial was performed. First, MolYsis Complete5 kit was applied. With the help of this kit the following is carried out in succession: a) all host blood cells in the sample collapse (when this occurs, the majority of non-host cells are not affected); b) non-host cells are isolated from the sample (whereas practically all contents of host cells are removed); c) non-host cells collapse, and from them nhDNA is isolated.

Next, for the samples received through such preparation, additional enrichment of nhDNA by decreasing hDNA concentration (NebNext microbiome enrichment) was performed. Then, WMS-test was carried out and reads belonging to hDNA (79%) were analytically excluded; other reads were mapped to within species with the use of NCBI Genome. They succeeded in mapping only 0.07% of reads. nhDNA of bacteria, viruses and fungi in correlation with the patients’ condition was discovered.

In (Gosiewski 2017) 62 patients with sepsis and 23 HP were examined, by 16S-test whole blood was studied, and information about bacDNA representation to within genus was obtained. The technique of DNA isolation from whole blood was based on their own research (Gosiewski 2014). Extra tests with samples of NTC (no template control) were carried out, which demonstrated composition of contamination (pollution of samples and/or reagents).

In (Kowarsky 2017) a large group of transplantation patients (heart, lungs, bone marrow) as well as 32 pregnant women (188 patients in total) were examined. Blood sampling was carried out repeatedly at...
different stages of transplantation and pregnancy (1351 samples in total). WMS-test was applied to study plasma (extracellular, circulating) DNA. There are no published data on its concentration. The emphasis in the research is placed on detecting and studying not mapped nhDNA (i.e. such DNA which cannot be compared to any known genomes of bacteria, archea, viruses, etc.).

95% of reads underwent quality control, and out of these 99.55% on average were mapped on reference human genome (GRCh38), i.e. only 0.45% on average remained for mapping on non-host reference. Only averagely 1% of not excluded were mapped on reference containing genomes of nearly 8000 species of known bacteria, archea, viruses, fungi and other eukaryotes (i.e. 0.0045% on average of all reads). About 1800 species (out of nearly 800 genera) were found in all blood samples.

Chart SF15 (Kowarsky 2017) shows quantitative characteristic of the mapped at the level of domains and phylums: Bacteria (528 in total), including such phylums as Actinobacteria (248), Firmicutes(183), Proteobacteria (86) and Deinococcus-Thermus (2); Eukaryota (145 in total), including such phylums as Ascomycota (96), Chordata (7), Bacillariophyta (5) and Streptophyta (4) and Viruses (100 in total). More detailed information (for example, on genera or species) is absent from the paper and appendices.

The results in (Kowarsky 2017) are distinctly different from (Grumaz 2016), primarily in a very high proportion of reads mapped on human genome (99.55% against 96-98%) and also in a lower proportion of reads mapped on non-host reference (0.0045% against 0.064% of all reads on average).

It might be connected with the process of blood plasma isolation. According to (Kowarsky 2017) it occurred by more intensive centrifugation (1600g, 10 min. + 16000g, 10 min.) while in (Grumaz 2016) it was only (292g, 10 min. + 1000g, 5 min.), which led to the removal of most large nhDNA fragments from plasma.

In this study manifold contamination control was applied. More specifically, some samples of NTC were formed of hDNA received from definitely sterile cultures of human cells. A similar approach to forming sample of NTC will be implemented in this project (Peslyak & Korotky 2019).

In (Panaiotov 2018) healthy persons are examined (28 people, with all the blood groups equally represented). Whole blood was cultivated with a special technique for low concentrations. All the samples demonstrated cultural growth. 16S-tests were carried out both for cultivated and initial samples of whole blood. For the first time fungal metagenome was identified by ITS2. DNA isolation was performed by the researcher’s own method with the subsequent use of two standard kits. bacDNA concentration was not determined; distilled water was used as control samples. Taxons found in control samples were eliminated from metagenome of the main samples.

In initial samples of whole blood the maximum representation was demonstrated by Rhizobiales and Sphingomonadales orders (over 90% in total), representation of Bacillales order amounted to appr. 2.3%. The results at the level of families and genera are not given in the study, though the accuracy of 16S-test enables us to do so. The results on whole blood metagenome do not correlate with the results in (Li 2018), they are not so exact and not analyzed thoroughly enough.

In (Puri 2018) patients with alcoholic syndrome of different severity (n=56) and HP control group (n=20) were examined. The method of DNA isolation from whole blood was the same as in (Païssé 2016). bacDNA concentration was subsequently determined and 16S-test was applied. bacDNA concentration for HP amounted to 66 (16S copies)/(DNA ng) on average (fig.1A, Puri 2018). NucleoSpin Blood kit was applied for DNA isolation from blood (average DNA yield amounted to 25000 ng/ml). It is therefore possible to estimate bacDNA concentration on 1 ml of whole blood in 1.65*10^6 (16S copies)/ml = 66 (16S copies)/(DNA ng) * 25000 ng/ml. It is less than 4.2*10^7 (16S copies)/ml on average for postprandial whole blood in (Païssé 2016), probably because blood sampling was carried out on an empty stomach. From the information of HP whole blood metagenome it follows that representation of Streptococcaceae family amounted to ~2.8% (Puri 2018, suppl. fig.3A).

In (Li 2018) patients with pancreatitis (n=50) and HP control group (n=12) were examined. Metagenome was studied by 16S-test, whole blood as well as previously isolated blood neutrophils were used as biomaterial. bacDNA concentration was determined only in whole blood and for 12 HP averaged 1.38*10^6 (16S copies)/ml. Blood sampling was carried out on an empty stomach and blood was immediately (before DNA isolation) processed by RLT buffer (Qiagen). Mechanical homogenization (similar to Païssé 2016 and Puri 2018) was not applied. Right after processing by RLT buffer, QiAamp DNA Mini Kit (Qiagen) was applied.

Blood metagenomes (both of whole blood and of isolated neutrophils) of patients and control group are analyzed in detail at the level of phylums, classes, families and genera. It is demonstrated that 80-90% of blood metagenome are constituted by the bacteria present in fecal metagenome. The authors suggest that intestine microbiome is the main source of bacterial product income into systemic blood flow.

It may be assumed that the highest bacDNA concentration in HP whole blood (compared to other studies) is achieved due to the immediate use (right after blood sampling) of RLT buffer. Its use proved to be more effective (compared to mechanical homogenization) not only for disrupting blood cells, but also for delaying bacDNA degradation processes.
In (Qian 2018) two groups of patients with Parkinson's disease were examined. For the first group (n = 45), their spouses (n=45) were selected as HP control group. One of the criteria to be selected for the first group was continuous residence in the given region for at least 20 years. Such restrictions were not applied for the second group of patients with Parkinson’s disease (n=58) and for HP control group (n=57). Having a meal before blood donation was not stipulated for patients and HP, i.e. for each specific patient it is unknown whether blood sample is after fasting or postprandial (information from the authors of the study). DNA isolation was made from leukocytic mass (similar to Amar 2011 and Amar 2013). bacDNA concentration in leukocytic mass was determined only for the first group of patients and HP. As well as in (Puri 2018), it was determined in the form of 16S copy quantity found in 1 ng of all DNA. Average DNA yield amounted to 15000 ng/ml of whole blood (information from the authors of the study). bacDNA concentration for HP averaged 7.78*10^3 16S copies on 1 ng of all DNA (Qian 2018, correct Tab.2), which corresponds to averagely 1.17*10^8 (16S copies)/(ml of whole blood).

Whittle 2019 studies the blood of 5 patients with pre-existing asthma and of 5 HP. Plasma was used as biomaterial for culturing and DNA isolation. The time of blood sampling is not indicated. The culturing tested positive for 8 samples (4 patients and 4 HP). Reducing contamination level during venipuncture was ensured by eliminating the first test tube. Ultraclear water (molecular biology grade water) was used as NTC. The authors consider intestine, oral and skin microbiomes to be the main sources of live bacteria and bacterial product income into blood.

Shah 2019 studies blood of patients with chronic renal failure and HP. The blood was taken from 2010 to 2017 and stored in a cryobank. As a result of detailed study of patient records, blood samples of 20 patients and 20 HP were selected. The (Païssé 2016, Puri 2018) technique was applied; leukocytic mass was used as biomaterial for DNA isolation. The time of blood sampling is not indicated; concentration was identified as the number of 16S copies found in 1 ng of all DNA. For 20 HP bacDNA concentration averaged 122 (16S copies) / (DNA ng), which corresponds to appr. 3.0*10^8 (16S copies) / (ml of all blood). This is almost twice as high as in (Puri 2018) and 10 times lower than in (Païssé 2016) for 30 HP. That is probably due to the fact that sampling of blood, stored in the cryobank, was performed on an empty stomach. Contamination level was not evaluated in this study.

Qui 2019 studies blood of 50 patients with type 2 diabetes and 100 HP. Plasma was used as biomaterial for DNA isolation. Blood sampling was carried out on an empty stomach; bacDNA concentration was not determined. The main part of blood metagenome was made by two classes: Alphaproteobacteria (average representation for HP ~56.9% and for patients ~56%) and Betaproteobacteria (40.8% and 41.7% respectively), including two genera, Sphingomonas (51.6% and 51.1%) and Variovorax (36.9% and 37.3%) (Qui 2019, tables S2 and S5).

This is essentially different from the results in Païssé 2016 (respective classes constitute 54.9% and 21.9%, respective genera – below 1%) and Li 2018 (respective classes – 9% and 7%, respective genera – below 1%). Sphingomonas and Variovorax genera with representation as in Qui 2019 were not previously found in blood and skin metagenomes of HP, and if they were, that was only in some samples and in a relatively small percentage (Oh 2014, skin metagenome, under 10% of sites, representation lower than 3%). Qui 2019 does not say anything about measures of reducing contamination by skin commensals during venipuncture. It can be assumed that blood from the first test tube was used for DNA isolation, whereas these two genera are the most widespread skin commensals of palmar forearm of the local population.

In Serena 2019 metagenomes of whole blood and fecal metagenomes were identified for 18 patients with celiac disease and 10 HP. Comparison of metagenomes was carried out. Concentration was not determined; measures to reduce and control contamination are not indicated. In HP blood metagenome, the following phylums demonstrated maximum representation: Proteobacteria (42.3%), Firmicutes (32.1%), Actinobacteria (8.4%) and Bacteroidetes (5.9%) (Serena 2019, fig.1A). The authors register a considerable decrease of genus Bifidobacterium representation at patients (under 10 OTU on average) compared to HP (300 OTU on average), which correlates with the information on the positive role of this genus’ bacteria in intestine microbiome as well as on its possible role in preventing and reducing celiac disease severity. The authors regard intestine microbiome as the main source of bacterial product income into blood, and hence they believe that blood metagenome can to a large extent characterize intestine microbiome composition.

In Cho 2019 blood metagenome was identified for 324 patients with cirrhosis or carcinoma of liver, and for 402 HP. Serum was used as biomaterial, and notably it underwent specific preprocessing before DNA isolation (including 40-minute boiling at 100°C). bacDNA concentration was not determined; measures to reduce and control contamination are not indicated. In HP blood metagenome the following phylums demonstrated maximum representation: Firmicutes (~40%), Proteobacteria (~26%), Actinobacteria (~12%), Bacteroidetes (~7%), Verrucomicrobia (~1.5%), and including genus Pseudomonas (10.1%), Streptococcus (5.8%), Bacteroides (~4.2%), Bifidobacterium (4%), Acinetobacter (2.2%), Staphylococcus (2%), Klebsiella
(2%), Enterococcus (2%), Faecalibacterium (~2.2%), Akkermansia (1.9%) and Prevotella (1.2%) (Cho 2019, fig.3 and tab. S1). The authors point out that representation of genera Bifidobacterium and Streptococcus at HP was considerably higher than at patients. HP selection was carried out only on the basis of assessing their state of health at the time of blood sampling, whereas no questions were posed about taking antibiotics or any other drugs potentially influencing blood metagenome. Comparing blood metagenomes of patients and of HP has made it possible to reveal a number of essential differences. They are regarded as the result of patients’ intestine microbiome changes.

In Dong 2019 blood metagenome was identified for 101 samples (88 patients with gastric carcinoma and 13 HP). Only these sample results out of 311 samples were kept for analysis after sequencing (261 patients and 50 HP). Low quality of sequencing results is mentioned as the main reason for this. Serum of blood taken on an empty stomach was used as biomaterial. bacDNA concentration was not determined; measures to reduce and control contamination are not indicated. In HP blood metagenome the following phylums demonstrated maximum representation: Proteobacteria (60.8%), Actinobacteria (25.8%) and Firmicutes (10%), including genus Pseudomonas (10.8%), Sphingomonas (~10%), Propionibacterium (~9%), Corynebacterium (~7%), Hydrogenophilus (~5%), Geobacillus (~5%), Acinetobacter (~3%) (Dong 2019, fig.2d,c).

A recent extensive review in (Castillo 2019) “The Healthy Human Blood Microbiome: Fact or Fiction?” is a retrospective review of studying the presence of bacterial products and live bacteria in HP blood. The authors analyze early works where culturing was applied as well as recent works where 16S-tests were also carried out (including many of the listed above).

The main conclusion made by the authors is that the presence of bacterial products (including bacDNA) in blood of HP is normal. The authors suppose that there is always a certain amount of live bacteria in HP blood. They consider intestine microbiome to be the main source of their income (as well as that of bacterial products).

Table 1 contains a brief description of the researches listed above. Note should be taken that bacDNA concentration in whole blood (leukocytic mass) in HP was determined only by 16S-test and only in four of the studies mentioned above (Table 2). There is a wide scatter of results. This can be partly explained by the following variations: by preparation for blood donation (obligatory meals for donors, 12-hour fasting for most groups of patients); by fractions (whole blood, leukocytes, buffy coat); by methods of DNA isolation (Psifidi 2015); by algorithms of read processing; by methods of measurement (assessment) of bacDNA concentration. Safety of nhDNA (including bacDNA) contained in blood phagocytes at the time of blood sampling is definitely affected by the storage and transportation time and conditions, as well as pretreatment before DNA isolation. Blood phagocytes (until they are destroyed) continue degradation of earlier endocyted non-host products (including nhDNA). The rate of this degradation depends on many factors (transport environment, temperature, etc.).

Contamination can also affect sequencing results. Contamination can occur during venipuncture, from reagents or from the medium in which tests are carried out. It can come from the performer, it can be accidental, etc. Thus the results in Qui 2019 confirm the necessity of eliminating the first test tube (and ideally the second one as well!) during venipuncture.

Using whole blood as biomaterial instead of plasma is also preferable since bacDNA concentration in whole blood exceeds its concentration in plasma by more than three orders (Païssé 2016). The fact remains that identical accidental (or inevitable) contamination for whole blood samples results in an error which is three orders lower than for plasma samples.

For more detail about contamination and ways of its control and record (Salter 2014, Glassing 2016, Hornung 2019).

In the table, for each work there is a note on whether contamination control was exercised (if there is no relevant information in the paper, it is presupposed that there was no such control) (Table 1).

3. bacDNA concentration in healthy whole blood

We have collected all published statistically significant results of researches in which bacDNA concentration in whole blood of healthy people was determined (Table 1, Table 2).

For the project it is important to achieve the greatest possible bacDNA concentration and, consequently, the highest bacDNA representation in all DNA isolated from whole blood. As a result, after enrichment performance by NebNext Microbiome Enrichment (NME) bacDNA representation will become even higher and, consequently, percentage of nhDNA reads in output (after WMS-test) will increase.

As it follows from the series of works by French researchers (Amar 2011, Amar 2013, Païssé 2016, Lelouvier 2016), the maximum bacDNA concentration in whole blood is achieved in the postprandial term.
However, neither breakfast menu nor blood sampling time after breakfast in Païssé 2016 are specified, bacDNA concentration in whole blood before and after food intake for the same patients was not compared. Therefore, several studies devoted to measuring LPS (and other substances) concentration in blood after food intake (Bala 2014, Erridge 2007, Ghanim 2010, Gnauck 2016a, Milan 2017, Munford 2016). Dynamics of postprandial bacDNA concentration in whole blood is similar to dynamics of postprandial LPS concentration (measured in plasma, though) (Bala 2014). In the performance of project’s tasks statement and results of these researches will be taken into account.

bacDNA concentration in whole blood is affected by preprocessing, namely by how soon after blood sampling the degradation processes of bacterial products in and out of blood cells will be terminated. For this very reason in (Païssé 2016, Puri 2018) triple mechanical homogenization was applied. However (judging by the results), the use of RLT buffer (Li 2018) is desirable, which made it possible to achieve higher bacDNA concentration in fasting blood compared to postprandial blood (Païssé 2016): 1.38*10^8 against 4.2*10^7 (16S copies)/(ml of whole blood).

4. Conclusion

Let us enumerate the main drawbacks present in some of the researches listed above. Without determining total bacDNA concentration, comparing metagenomes of patients and healthy people becomes virtually pointless. Comparing several examinees’ metagenomes makes sense only if their total bacDNA concentrations differ very little.

If determining total bacDNA concentration has been done, in the absence of contamination control there is still a problem of reliability of the received results, since it still remains unknown for which bacDNA concentration is considerably higher than contamination level (these values are significant) and for which bacDNA concentration does not exceed contamination level (these values are insignificant).

Païssé 2016 demonstrated for the first time that bacDNA concentration in whole blood (leukocytic mass) is three orders higher compared to its concentration in plasma. However, in most studies of blood metagenome carried out after the publication of this work, only plasma (or serum) metagenome was identified. Thus values of bacDNA concentration were considerably closer to contamination level in absolute values.

In the same study, bacDNA concentration in postprandial blood was determined for the first time. There is every reason to suppose that it is significantly higher than in fasting blood (at least one order higher). Studying metagenome of postprandial whole blood makes it possible to improve the reliability of information on minor components of blood metagenome, primarily due to reducing the influence of contamination.

Reducing contamination level and reliable evaluation of its level necessitate elaboration of research protocol, including sampling, storage and transportation of biomaterials as well as including a sufficient number samples of NTC in the protocol (at all its steps).

In several studies contamination composition was determined, but its level was not estimated. Such an approach is irrational as merely knowing contamination composition does not enable us to consider its influence on metagenome of the main samples. It is a mistake to eliminate any bacDNA from metagenome of the main samples just because they were found in samples of NTC. Information on bacDNA concentration both in the main samples and in samples of NTC is indispensable for correcting metagenome of the main samples.

This review is made as part of preparation of NCS1 project (Peslyak & Korotky 2019) in which the main hypotheses of systemic YN-model of psoriasis pathogenesis will be tested (see Supplement S3).

Table 3 demonstrates the novelty of NCS1 project. Up to the present, blood metagenome of PP has been studied only by 16S-test (plasma and serum) (Munz 2010, Codoner 2018) whereas metagenome of PP whole blood has never been identified. In (Okubo 2002) only monocytes were studied, notably without metagenome identification. The method of whole genomic sequencing (WMS-test) has never been applied to studying metagenome of whole blood.

On the basis of the results of implementing NCS1 project, for the first time answers to the following questions will be received:

- Does severity of psoriatic disease correlate with concentration of any nhDNA in whole blood?
- Does severity of psoriatic disease correlate with any PAMP concentration in blood?
- Does severity of psoriatic disease correlate with increased macromolecular small intestine permeability?

To achieve this, for the first time (for PP and HP) (Table 3):
Parameters of fragment distribution of bacDNA, found in DNA-samples from whole blood are determined.
- Whole blood metagenome is identified by whole metagenomic sequencing method.
- nhDNA concentration in whole blood is determined (for the first time for PP).
- Macromolecular small intestine permeability is determined by bacDNA-test.

In the process of preparing and implementing the project, measures will be taken to minimize contamination level. Using samples of NTC will enable us to obtain complete and reliable information about the level and composition of contamination. This information will be considered for correcting metagenome of the main samples. Using WMS-tests will make it possible to determine metagenome composition to within species (and, if need be, to within strains). This will enable us to answer the questions raised within the project with sufficient reliability.

5. Supplements
S1. Comparative characteristics of 16S and WMS-tests.
S2. Resources of metagenomic research and sequencing.
S3. YN-model of psoriasis pathogenesis. Partial description.

6. Abbreviations and terms

| Abbreviation | Description |
|--------------|-------------|
| bacDNA       | Bacterial DNA |
| hDNA         | Host DNA (here - human DNA) |
| HP           | Healthy person |
| LPS          | Lipopolysaccharide |
| Metagenome   | Set of all nhDNA (non-host DNA, i.e. in this project of non-human), contained in biomaterial. |
| nhDNA        | Any non-host DNA (here non-human) – from bacteria, archean, fungi, plants, helminths, viruses, phages, etc. |
| NTC          | No Template Control. Control samples which do not contain DNA what is supposed to be found in the main samples. Are applied to determination of contamination level and concrete contaminants. |
| PAMP         | Pathogen-associated molecular patterns. (in particular LPS, PG, bacDNA) (Fukui 2016) |
| PG           | Peptidoglycan. |
| PP           | Psoriatic patient |
| Representation | Relative presence (percentage share) something in biomaterial (sample). |
| WMS          | Whole metagenome sequencing (shotgun) |

Bold indicates common abbreviations.

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### Tables

#### Table 1. Blood metagenome researches. Report.

| Patients (psoriatic patients) and HP | Biomaterial | Test | Determining concentration | Contamination control | nhDNA (particularly bacDNA) | Study (year, country), notes, reference to bioproject |
|-------------------------------------|-------------|------|---------------------------|-----------------------|-----------------------------|-----------------------------------------------------|
| 19 PP                               | Plasma      | 16S  | no                        | yes                   | bacDNA in 9 out of 19 PP    | Wang 1999 (USA). PP with psoriatic arthritis. Metagenome was not identified. |
| 15 PP, 12 HP                       | Monocytes   | 16S, 18S | yes                      | yes                   | bacDNA in all.             | Okubo 2002 (Japan). Relative level in PP is 1.5-2 times higher than in HP. Metagenome was not identified. bacDNA concentration was estimated in reference to concentration of gene human glyceraldehyde-3-phosphate dehydrogenase (GAPDH). |
| 20 PP, 12 HP                       | Plasma      | 16S  | no                        | yes                   | bacDNA in all PP, in no HP | Munz 2018 (UK). Metagenome was not identified. |
| 54 PP, 27 HP                       | Serum       | 16S  | no                        | no                    | In 16 out of 54 PP, in no HP. | Ramírez-Boscá 2015 (Spain) - article, nuanced in Codoner 2018. |
| 52 PP                               | Serum       | 16S  | no                        | no                    | In 13 out of 52 PP.         | Codoner 2018 (Spain). Based on the same material as Ramírez-Boscá 2015. |
| Non-psoriatic patients and/or HP    |             |      |                           |                       |                             |                                                     |
| 50 HP                               | Leukocytic mass | 16S | yes                      | no                    | bacDNA in all              | James 2011 (Canada). Metagenome was not identified. |
| 3280 persons                       | Leukocytes  | 16S  | yes                      | no                    | bacDNA                     | Amar 2011 (France quantitative 16S. Qualitative 16S was used for 42 people only. Patient group common with (Amar 2013), including those with diabetes. |
| 3936 persons                       | Leukocytes  | 16S  | yes                      | no                    | bacDNA                     | Amar 2013 (France), concentration assessment only for Eubacteria and Proteobacteria phylum in general. Metagenome was not identified. Patient group common with (Amar 2011)), including those with cardiovascular diseases. |
| 80 patients and 40 HP              | Plasma      | 16S and WMS | yes                    | yes, NTC        | bacDNA in all              | Dinakaran 2014 (India), WMS-test was performed for 3 patients and 3 HP. QIAamp DNA blood mini kit was applied. |
| 50 patients, 50 HP                 | Plasma      | 16S RT-PCR | no                     | no                    | bacDNA in 14 out of 50 patients, in 2 out of 50 HP | Sato 2014 (Japan), patients with type 2 diabetes. Tests of blood plasma were carried out in addition to tests of feces. Limited set of primers was used. Metagenome was not identified. |
| 78 patients and 10 HP              | Plasma      | WMS  | no                        | no                    | nhDNA in all               | Long 2016 (China), post-surgery patients, all nhDNA was studied, culture was performed. |
| 30 HP                               | Whole blood and 3 fractions | 16S | yes, NTC, 0.05% | bacDNA in all | Piaisd 2016 (France), HP - donors (postprandial blood tests). Increased bacDNA concentration provided by (Lluch 2015) method. NucleoSpin Blood L was applied. |
| 2 groups of patients (Spain - 37, Italy - 71) | BC fraction and whole blood | 16S | yes                      | no                    | bacDNA in all              | Lelouvier 2016 (France), each of the two patient groups was divided into two parts (without fibrosis, with fibrosis). BC - Buffy coat. |
| 30 HP, 90 patients                 | Plasma      | WMS  | yes – for all DNA only   | yes (not published)  | nhDNA in all               | Grumaz 2016 (Germany), all nhDNA was studied, patients with sepsis and post-surgery patients. Culture was also performed. ENA: PRJEB13247 |
| 9 patients                         | Non-host cells in whole blood | WMS | no                        | yes                   | nhDNA in all               | Gyrarmati 2016 (Sweden), acute leukemia and suspected sepsis. Before sequencing, consecutive enrichment was performed (Molysis, NebNext). All nhDNA was studied. Concentration was determined only for cell-free DNA. |
| 62 patients and 23 HP              | Whole blood | 16S | no                        | yes                   | bacDNA in all              | Gosiewski 2017 (Poland), patients with sepsis, DNA isolation procedure is based on their own research (Gosiewski 2014). |
| Patients | Biomaterial | Test | Determining concentration | Contamination control | nhDNA (particularly bacDNA) | Study (year, country), notes, reference to bioproject |
|----------|-------------|------|---------------------------|-----------------------|---------------------------|---------------------------------------------------|
| 188 patients, 1351 samples | Plasma | WMS | no | Yes, NTC | nhDNA in all | Kowarsky 2017 (USA), patients after transplantation (heart, lungs, bone marrow) and pregnant women. Numerous blood sampling from the same patients under observation. NTC was formed of sterile culture of human cells. |
| 28 HP | Whole blood | 16S | no | yes, dH₂O | bacDNA and fungal DNA | Panaiotov 2018 (Bulgaria), culturing and comparing of whole blood metagenomes for different blood groups were carried out. |
| 56 patients, 20 HP | Whole blood | 16S | yes | | bacDNA in all | Pun 2018 (USA, France), patients with alcoholic syndrome, DNA isolation procedure is similar to Païssé 2016. |
| 50 patients and 12 HP | Whole blood, neutrophils | 16S | yes | yes | bacDNA in all | Li 2018 (China), patients with pancreatitis. For the first time metagenome of blood neutrophils is identified. |
| 45 patients + 57 HP and 58 patients | Leukocytic mass | 16S | yes | yes, NTC=6, 0.14% | bacDNA in all | Qian 2018 (China), patients with Parkinson’s disease. Results on concentration raise doubts. |
| 5 HP and 5 patients | Plasma | 16S | no | yes | bacDNA in all | Whittle 2019 (United Kingdom), patients with asthma, culturing, RNA-seq. |
| 20 patients and 20 HP | Leukocytic mass | 16S | yes | no | bacDNA in all | Shah 2019 (USA, France), patients with chronic renal failure, but without diabetes. technique of DNA isolation is similar to Païssé 2016. |
| 50 patients and 100 HP | Plasma | 16S | no | yes | bacDNA in all | Qui 2019 (China), patients with type 2 diabetes. |
| 18 patients and 10 HP | Whole blood | 16S | no | no | bacDNA in all | Serena 2019 (USA), patients with celiac disease. Fecal metagenome is also obtained, and its comparison with blood metagenome is made. |
| 324 patients and 402 HP | Serum | 16S | no | no | bacDNA in all | Cho 2019 (Korea), patients with cirrhosis or with liver carcinoma. |
| 88 patients and 13 HP | Serum | 16S | no | no | it is not clear | Dong 2019 (China), patients with gastric carcinoma. |
| NCS1 research project | | | | | | |
| 30 PP, 10 HP | Whole blood | WMS | yes | yes | nhDNA in all | Peslyak & Korotky 2019, Russia, blood sampling 3 hours after food intake, preliminary elimination of hDNA, all nhDNA. Similar tests (of phagocytes) of psoriatic biopsies. Their complex study. |
Table 2. bacDNA concentration in HP blood. Report.

| Study | Okubo 2002 | James 2011 | Païssé 2016 | Puri 2018 | Li 2018 | Qian 2018 | Shah 2019 |
|-------|------------|------------|-------------|-----------|---------|-----------|-----------|
| Country | Japan | Canada | France | USA, France | China | China | USA, France |
| Biomaterial | Monocytes | Leukocytic mass | Whole blood (and other fractions) | Whole blood | Whole blood | Leukocytic mass | Leukocytic mass |
| HP (healthy persons) and patients | 12 HP and 15 PP | 50 HP (donors) | 30 HP (donors) | 20 HP (and others) | 12 HP (and others) | 45 HP (and others) | 20 HP (and others) |
| Time of blood sampling | ND | ND | after drink and meal | ND | after fasting | without control | ND |
| Preprocessing of samples before DNA isolation | Homogenization | Homogenization | RLT buffer | Phenol / chloroform | Homogenization |
| Whole blood. (16S copies)/(ml of whole blood) | 4.29*E7 (recalculation) | 4.20*E7 (recalculation) | 1.65*E8 (recalculation) | 1.38*E8 (recalculation) | 1.17*E8 (recalculation) | 3.05*E6 (recalculation) |
| Determination of bacDNA concentration (qPCR) | V3, special primers, 194 - amplicon | V3-V4, EUBF, EUBR, 467 - amplicon | V3-V4, universal primers, ND | V3, 357/518r, 162 - amplicon | V3-V4, EUBF, EUBR, 467 - amplicon | qPCR (V3-V4, EUBF, EUBR, 467 - amplicon) |
| average bacDNA E.coli weight proportion from all DNA in whole blood (recalculation) | 0.022% | 0.060% | 0.059% | 0.002% | 0.194% | 0.164% | 0.004% |
| (16S copies)/monocyte (Okubo 2002) | HP - 3.1; PP - 5.8 | 5.7 |
| Concentration range | HP: 1.5 - 7.5 | 0 - 0.48% (in % of weight) | 1.8E7 - 7.6E7 | +.50% | 8E7 - 2E8 | ? | Q1=2.9E6, Q3=3.7E6 |
| Contamination control | NTC | NTC >=5 | NTC=6 |
| Contamination level | 0.048% (average) | 0.003% (maximum) | 0.138% (average) |

Table 3. Blood metagenome researches. Fact and plan.

| 16S-test (PP) | 16S-test (HP) | WMS-test (PP) | WMS-test (HP) |
|---------------|--------------|---------------|---------------|
| Plasma, serum | Munz 2010; Codoner 2018 | Païssé 2016 (+) and others | Dinakaran 2014 (+); Long 2016; Grumaz 2016 (+); Kowarsky 2017 (HP – pregnancy); |
| Whole blood, phagocytes | Okubo 2002 (monocytes – without metagenome, +); Païssé 2016 (and its fractions, +); Gosiewski 2017; Puri 2018 (+); Li 2018 (including neutrophils, +); Qian 2018 (leukocytic mass, +); Shah 2019 (leukocytic mass, +). | NCS1 (+) | NCS1 (+) |

Note: + - concentration was (will be) determined.
Intestine microbiome self-renews; as a result of bacterial activity and death of bacterial cells, bacterial products develop, particularly LPS (lipopolysaccharide), cellular wall component of Gram(-) bacteria and PG (peptidoglycan), the main cellular wall component of Gram(+) bacteria, as well as bacDNA (bacterial DNA). At normal, and especially at increased small intestine permeability for bacterial products, their essential part gets into systemic blood flow.

At least 95% of venous blood from small intestine passes by portal vein through liver, where an essential part of bacterial products is degraded and filtered (and subsequently returns into small intestine together with bile). Due to porto-caval anastomosis, though, up to 5% of venous blood bypasses liver and gets directly into the system of superior vena cava, and then into systemic blood flow (including non-degraded bacterial products).

In systemic blood flow, bacterial products are constantly utilized, mainly with phagocyte participation (primarily neutrophils). The figure shows both young phagocytes (which have just left bone marrow and therefore do not contain bacterial products) and those which have already endocyted bacterial products. At each timepoint most non-degraded bacterial products in systemic blood flow are either bound by phagocytes through surface receptors or are endocytosed and located in phagocytes.
## Supplements

### S1. Comparative characteristics of 16S and WMS-tests

| Characteristics                                                  | 16S                | WMS                | Sources                          |
|------------------------------------------------------------------|--------------------|--------------------|----------------------------------|
| **Basic**                                                       |                    |                    |                                  |
| 1 Opportunity to detect any species, including uncultivated ones.| Yes                | Yes                |                                  |
| 2 Identifying DNA of both live and dead (partly degraded, including degraded DNA) organisms. It is impossible to identify separately. | Yes                | Yes                | Jovel 2016, Meisel 2016, Ranjan 2016 |
| 3 Detecting non-bacterial DNA (eukaryotes, archean, viruses, phages, fungi, plants, parasites, etc.). | No                 | Yes                | Jovel 2016, Meisel 2016, Ranjan 2016 |
| 4 Level of high-quality taxon classification.                   | Phyla +++          | Phyla +++          | Frey 2015, Meisel 2016, Tyakht 2014 |
| 5 Representation (phylums, genera, species) is determined.      | Yes                | Yes                | Bhat 2016, Glassing 2016, Grumaz 2016, Païssé 2016, Tan 2015 |
| To determine concentrations by representations, an additional test of initial biomaterial is carried out. qPCR (Glassing 2016, Païssé 2016) and dPCR (Bhat 2016, Tan 2015) are most often applied to determine total bacDNA by one of the universal 16S rRNA sites (Nakatsuji 2013). "The internal standard method" – adding a specific amount (about 1% of the totally expected amount) of DNA of a characteristic bacterium to biomaterial – is also applied. It is such a bacterium whose bacDNA definitely cannot be present in this biomaterial (Tan 2015). Before sequencing, concentration of all DNA is always determined. This can be enough to determine concentration by WMS-test results. |
| 6 Genome coverage. For WMS-test, possible even for DNA with low representation (depends on coverage depth). | One or several sites in 16S rRNA | Uniform coverage of all genome. | Ranjan 2016 |
| 7 Identifying specific genes. Due to this, WMS-test results can be interpreted with precision up to within strains on marker unique genes (Jovel 2016). | No                 | Yes                | Ferreti 2017, Ranjan 2016         |
| 8 Identifying genes of resistance to antibiotics (resistome).   | No                 | Yes                | Frey 2015                         |
| 9 Identifying genes of virulence (pathogenicity).               | No                 | Yes                | Meisel 2016                       |
| 10 Functional classification of the detected species, discovery of new genes. | No                 | Yes                | Jovel 2016                        |
| 11 Information for the choice of medicines.                     | +                  | ++                 | Frey 2015                         |
| Characteristics                                                                 | 16S | WMS | Sources                  |
|--------------------------------------------------------------------------------|-----|-----|--------------------------|
| **Additional characteristics and disadvantages**                                |     |     |                          |
| 12 Quantity of genera (species) of bacteria for which 16S rRNA sequence (fully or partly, for 16S-test) or strain genome sequence (for WMS-test) is established. Data for Feb 14, 2019. | ~ 3360000 (RDP, r11); ~ 6800000 (SILVA, r132) | ~ 186000 (including ~13500 whole Genome) |                          |
| 13 Quantity of bacteria species (for 16S-test with inaccurate classification) found in one biomaterial (excrements) by increasing the size of libraries (up to 3.2*10^7). | 2050 | 4100 | Ranjan 2016              |
| 14 Microbiome variety found in one biomaterial on three various metrics.       | Lower | Higher | Ranjan 2016              |
| 15 Test-sensitivity is limited by possible contamination. Test samples of NTC are necessary to assess contamination level. It is also essential to take constant measures to reduce this level. | Yes | Yes | Glassing 2016            |
| **WMS-test vs 16S-test.**                                                      |     |     |                          |
| 16 When the pathogen is unknown, when more than mere identification is required (identifying the strain, assessing pathogenic load and resistance to antibiotics). In case of mixed infections (e.g. at mixed sepsis), 16S-test often results in mistakes and has weak repeatability. | No | Yes | Frey 2015                |
| 17 Opportunity to detect DNA of any species, and not only that which is included into a pre-determined list. The best 16S tests of sepsis diagnostics SepsiTest (over 345 species of bacteria and fungi) and IRIDICA (over 1000 pathogens, out of production since 2017) are unable to identify pathogen presence, if it is not from their list. | No | Yes | Frey 2015, Stevenson 2016 |
| 18 Identifying genomes of new, previously undetected species.                  | No  | Yes | Frey 2015, Jovel 2016    |
| 19 **16S-test. Disadvantage.** The number of 16S copies in genome changes within a wide range (depending on species and even strain). It results in assigned errors when determining taxon representation. Representation of taxons with a larger number of 16S copies in genome will be overestimated, with a smaller number – underestimated. | Yes |     | Vetrovsky 2013, Tyakht 2014 |
| 20 **16S-test. Disadvantage.** The choice of primers for different variable sites (from V1 to V9) to perform amplification, leads to essentially different results not only due to their different characteristics at amplification (affinity), but also due to influence on classification by taxons. | Yes |     | Jovel 2016, Meisel 2016   |
| 21 **16S-test. Disadvantage.** Mutations in variable sites 16S rRNA (from V1 to V9) can interfere with the correct classification by taxons. | Yes |     |                          |
| Characteristics | 16S | WMS | Sources |
|----------------|-----|-----|---------|
| **WMS-test. Disadvantage.** The necessity of maximum elimination of host DNA (hDNA) from biomaterial before sequencing (biochemical methods) and after sequencing (algorithmic methods). | | Yes | Ferretti 2017, Frey 2015 |
| **Cost performance and specific performance data** | | | |
| Cost at the rate of one sample. Depends on problem statement, number of samples in library, sequencer power and its working mode, protocol of processing sequencing results. | 47-60 $ | 120-290 $ | Ranjan 2016, Genohub, Allseq |
| **WMS-test.** In the future, the cost may fall to below $1 per one bacterial genome (2014). It has already happened (see above). The cost can be reduced due to sample preparation or by enrichment of pathogen representation and/or host DNA elimination. | | Yes | Applications 2015, Frey 2015 |
| Performance time (depends on equipment and problem statement) | 2-5 ч | 7–60 ч | Frey 2015 |
| Requirements to temperature control during transportation and sample preparation. | Lower | Higher | Frey 2015 |
| Proven pipeline of test implementation. Term of active use. For 16S-test, however, there are no (and cannot be any) satisfactory schemes for classification to within species (Jovel 2016). | A lot. Over 30 years. | Few. About 10 years. | Sharpton 2014, Ranjan 2016, Nayfach 2016, Vincent 2017, Aransay 2016 (part 12) |
| Number of publications “16S + metagenomic” vs “shotgun + metagenomic” (according to https://scholar.google.com/) | 50800 | 24300 | 14.02.2019 |
## S2. Resources of metagenomic research and sequencing

| Title | Description. Notes |
|-------|--------------------|
| **HMP (Human Microbiome Project)** | All information about microorganisms, living on and in human body (the project was founded in 2008), contains information on more than 3,000 genomes. |
| **KEGG** | Kyoto Encyclopedia of Genes and Genomes (over 4,000 genomes) |
| **MetaHIT** | Intestine microbiome. The project was completed in 2012. |
| **Integrated gene catalog (IGC)** | Catalog of intestine microbiome genes |
| **NCBI Reference Sequence (RefSeq) Database** | NCBI. Reference DB of genomes. Statistics. |
| **NCBI.Genbank** | NCBI. DB of genomes ~ 186000 genomes of prokariot, including ~ 13500 whole ones. |
| **NCBI Microbial Genomes Resources** | NCBI. DB of bacterial genomes. Taxonomical tree. |
| **NCBI. Sequence Read Archive** | NCBI. DB of metagenomic projects. |
| **Genomes OnLine Database** | DB of genomes. |
| **MG-RAST** | DB of metagenomic projects. |
| **Allseq. The Sequencing Marketplace** | Information on sequencers. |
| **Genohub** | Information resource on sequencing methods and provider choice. Search of provider. |
| **Science Exchange** | Information resource on sequencing methods and provider choice. Search of provider. |
| **Omictools** | Search of software for processing biological (including metagenomic) research results |
| **Center for Genomic Epidemiology** | Resource for infectious disease specialist |
| **The European Bioinformatics Institute. Metagenomics** | DB of metagenomic projects. |
**S3. YN-model of psoriasis pathogenesis. Partial description.**

**Fig. S1. YN-model of psoriasis pathogenesis.** (Peslyak & Korotky 2019, part 5.2).

**IB-Y**  
Interpeptide bridges of peptidoglycan Str.pyogenes: (L-Ala)-(L-Ala) or (L-Ser)-(L-Ala).

**PG-Y**  
Peptidoglycan A3alpha with interpeptide bridges IB-Y (but can also contain others bridges)

**PsB**  
Psoriagenic bacteria - species of bacteria presumed psoriagenic (with PG-Y peptidoglycan)

**Y-antigen**  
part(s) of interpeptide bridge IB-Y

**SIBO**  
Small intestine bacterial overgrowth. Excess of total bacteria concentration over norm and/or pathogens presence in biomaterial. Smears, scrapes from mucosa or aspirates can be used as biomaterial.

**Systemic psoriatic process**  
SP1. Increased small intestine permeability for PAMP (including LPS, PG, bacDNA).

SP2. SIBO with PsB-bacteria.

SP1 and SP2 result in chronically increased  
- concentrations of PAMP (including PG-Y) in blood flow;  
- PAMP-(PG-Y)-load on blood neutrophils;

As a result, many blood neutrophils  
- become PAMP-(PG-Y)-carriers;  
- pass into prenetotic state;  
- undergo netosis.

**Local processes**  
In healthy skin neutrophils are practically absent. They are attracted from blood flow at the earliest stage of psoriatic plaque initiation (even before visible skin changes Their intensive attraction continues as long as the plaque is present. In stable or growing plaque, neutrophils end their existence due to pro-inflammatory environment, mostly by netosis (or, at plaque remission, by apoptosis).

Non-degraded PAMP (including PG-Y) brought from blood flow get into netotic products. They are endocyted by skin phagocytes, particularly by dendritic cells.

Dendritic cells process PG-Y and present Y-antigen (contained in PG-Y) to effector T-lymphocytes. Other PAMP act as adjuvants. False adaptive response of skin immune system to false PsB-infection is formed. Psoriatic plaques appear and grow while **systemic psoriatic process** is going on, i.e. while neutrophils still attracted from blood flow are abundant in PAMP and PG-Y.
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