Metagenomes of blood and psoriatic skin

Research project

Edition e2.2a

Mikhail Peslyak, Nikolay Korotky

Antipsoriatic Association
"The Natural Alternative"
Moscow, 2019

UDC 616.5:616-092;

Mikhail Yuryevich Peslyak[1], Nikolay Gavrilovich Korotky[2].
Metagenomes of blood and psoriatic skin. Research project. e2.2a edition, M.: Antipsoriatic Association "The Natural Alternative", 2019. - 67 pages.

ISBN 978-5-90-550406-8

[1] **Antipsoriatic Association "The Natural Alternative"**
[2] **Pirogov Russian National Research Medical University**

Electronic Publication Date of edition e2.2: 2019, May 7.
DOI of this edition: 10.5281/zenodo.2667680
DOI of latest edition: 10.5281/zenodo.2667679

Web: www.psorias.info, E-mail: info@psorias.info

The given book is the authorized translation of the book (edition r2.2), published in Russian (ISBN 978-5-905504-05-1, DOI: 10.5281/zenodo.1415418). Translation has been carried out with active support of "Status Translation" agency. Reprinting at occurrence of new materials or detection of serious errors is supposed. Further, updated texts (Russian and English) of the book will be placed in the Internet.
Details and links on www.psorias.info.

Main supplements

A. Metagenomes of blood and psoriatic skin. Research project. Presentation and illustrations. (e2.2, pdf-format, DOI: 10.5281/zenodo.2668376)

B. Metagenomes of blood and psoriatic skin. Research project. Supplements S1-S8. (e2.2, pdf-format, DOI: 10.5281/zenodo.2668459)

Abstract

The first stage of the project devoted to the study, diagnostics and treatment of psoriasis is substantiated and given a detailed description. The purpose of the first stage is testing the basic hypotheses of new systemic YN-model of psoriasis pathogenesis. The test consists in complex studying of whole blood metagenome and metagenome of psoriatic skin (phagocytes). Metagenome is all non-host DNA (here - non-human) which is found in biomaterial. The necessary algorithms for complex studying of metagenomes are suggested and substantiated.

Survey and analytical assessment of the results of fundamental works on studying metagenomes of blood and skin is carried out. All works (published prior to the beginning of 2019) 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.

The objectives and tasks (including the order of solving them) of the first stage are formulated. The main questions are raised, to which the results of the first stage are supposed to give answers. The order of preparing and implementing WMS-test of whole blood is suggested.

Detailed comparison of YN-model of pathogenesis with the previously published Y-model is conducted. An analytical review of works on studying the attraction of blood neutrophils in psoriatic skin and their possible subsequent netosis is made. Netosis in psoriatic skin of neutrophils which are carriers of endocytosed Y-antigen in blood is qualified as an essential link in the vicious cycle of psoriatic inflammation within YN-model. Classification of psoriatic disease as netopathy is suggested.

Psoriagenic bacteria are given definition based on the existence of genes responsible for the formation of interpeptide bridges. The smallest peptides (peptidoglycan fragments) - potential Y-antigens are identified.

Illustrations are made as a presentation and can be found in Supplement A (section 5.12).

Keywords

Bacterial DNA, bacterial products, bioinformatics, chemokines, dendritic cells, human papilloma virus, innate and adaptive response, intestinal permeability, keratinocytes, kPAMP-carriage, lipopolysaccharide, metagenome, microbiome, model of pathogenesis, monocytes, muramyl dipeptide, netosis, neutrophils, non-host DNA, PAMP-nemia, peptidoglycan, phagocytes, psoriagenic bacteria, psoriasis, psoriatic disease, sequencing, systemic psoriatic process, T-lymphocytes.

Content

Tables

1. Introduction

The preliminary version of the project (v1.6) was prepared and published earlier (Peslyak 2017a, Peslyak 2017b). Then we submitted the project at VII Interregional forum of dermatovenerologists and cosmetologists (Peslyak & Korotky 2017). Time which has passed since then has enabled us to conduct a more detailed project development and to take into account the results of recently published major works (Tett 2017, Codoner 2018, Horiba 2018, Li 2018, Loesche 2018, Marotz 2018, Puri 2018, Qian 2018). It has enabled us on the basis of Y-model to formulate new YN-model of psoriasis pathogenesis, including netosis in psoriatic skin as a link of the vicious cycle (section 5.2).

During project specification it became clear that it was inexpedient to include isolation of phagocytes from whole blood into the worksheet (section 2.1). Besides, the decision on the order of isolation of DNA from biopsies of psoriatic skin (directly from them, or from previously isolated phagocytes) would be taken during pilot stage (section 5.11).

It is due to this that the word "phagocytes" (which was present in the v1.6 version) has been deleted from the project title.

1.1. Psoriatic disease

Epidermis self-renewal is 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 (CPs) is the most frequent type (more than 80% of total number of cases) (slide *2. Psoriatic_disease*). 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.

Slides *3. Patient_Stat-C* and *4. Patient_Stat-R* present regional statistics of psoriasis incidence according to (Michalek 2017, part of table 3). It is supplemented by the US 2004 statistics for psoriatic patients (PP) over 18 (Vanderpuye 2015) (for full list of abbreviations see section 5.1). Russian data are cited from (Znamenskaya 2012, Mishina 2015). The economic data connected with the expenses on psoriasis treatment and losses on disability are regularly calculated in developed countries. In detailed research (Vanderpuye 2015) made for the USA it is stated that for 5.3 million PP over 18 living at the beginning of 2013 (about 2.2% of the population) the annual economic burden constituted $35.2 billion, out of which $12.2 billion was health care expenses, i.e. the annual expenses on one PP amounted to $6592, $2307 out of which was for health care. Other studies of this subject mention similar annual expenses on psoriasis treatment in the USA.

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. "*Federal clinical recommendations on psoriasis patient management*", accepted in Russian Federation (Federal 2015) give detailed recommendations on the use of topical drugs, phototherapies, systemic retinoid and immunosuppressors. The given recommendations are aimed at reducing the external displays of psoriasis, but not at eliminating the reasons for its initialization and support.

1.2. Models of pathogenesis and unknown antigen

There are several models of pathogenesis of psoriatic disease (PD), but none of them is universally accepted. The systemic BF-model (slide *12. BF-model*) and Y-model are described in (Baker 2006b) and (Peslyak 2012a, Peslyak 2012b) respectively. The local N-model (Perera 2012), GK-model (Guttman-Yassky 2011) and TC-model (Tonel 2009), and GL-model (Gilliet 2008) are considered and given a comparative analysis in the monograph (Peslyak 2012b). Local models by other authors (on the whole, similarly constructed) have been published in recent years. They include the specified GKH-model (Hawkes 2017); FM-model (slide 68. FM-model, Delgado-Rizo 2017); SE-model (slide 69. SE-model, Schon 2018) and BMM-model (slide 71. BMM-model, Benhadou 2018).

The modern idea of skin immune system functioning is outlined in the video "Immunology of Skin" directed by Miriam Merad and James Kruger (Supplement C, 5.12). Part two of the video deals with the wrong work of skin immune system during psoriatic inflammation. The key role of the "unknown antigen" (hereinafter Y-antigen) in psoriatic inflammation is also highlighted (slide *13. Antigen-1*). Subsequently, a number of new studies devoted to psoriatic disease were published. But none of them has yet given exact answers to the questions:

- What is the origin of Y-antigen?
- What is the chemical structure of Y-antigen?

Four versions of the origin of Y-antigen are submitted (Table 1, slide *14. Antigen-2*).

The question of the assumed chemical structure of Y-antigen is considered below (including section 5.5).

Table 1. Versions of Y-antigen origin.

Y-antigen is	Version status
Version A. Autoantigens descended from host resident skin cells.	All local models of pathogenesis presume that the main reasons for initiation and support of psoriasis are located only in skin (and, ncluding, unknown antigen). Such potential autoantigens were studied repeatedely (keratin 17, LL37, ADAMTSL5, PLA2G4D and others) (Arakawa 2015, Cheung 2016, Fuentes-Duculan 2017, Valdimarsson 2009). Specific to them T-lymphocytes are found in psoriatic skin, but not in all psoriatic patients. Therefore any of them cannot be Y-antigen, specific T-lymphocytes to which must be found in _each_ psoriatic patient. Version A is not proved.
Version B. Fragments of chemicals or bacteria, fungi, viruses or proteins secreted by them coming on or into the skin from external environment.	The given version already existed in the 20th century. For many years this version was considered as the main one. But numerous studies to prove version B inadequate (Baker 2008).
Version C. Fragments of chemicals or bacteria, fungi, viruses or proteins secreted by them. Come to psoriatic skin from other body organs (e.g. in blood phagocytes).	The main version of the authors of systemic models of pathogenesis: BF-model. Barbara Baker and Lionel Fry (slide *12. BF-model*, Baker 2006a, Baker 2006b); Y-model. Peslyak MY and Korotky NG. (Korotky 2005, Peslyak 2012a, Peslyak 2012b); YN-model (described for the first time in this project) (section 5.2, slides *22. SPPN-PAMP-nemia*, *23. SPPN*, *25. Local_processes_YN*, *26. YN-model*). The known facts do not contradict version C. Within the project several basic hypotheses, common for both Y-model and YN-model, will be tested.
Version D. Autoantigens descended from host non-resident cells. Coming to skin from other body organs (for example, fragments of phagocytes of blood).	It is part from potential autoantigens (LL37, ADAMTSL5, PLA2G4D) listed in version A description. They descend not only from resident skin phagocytes (neutrophils, dendritic and mast cells), but also and from non-resident phagocytes attracted from blood flow during psoriatic inflammation. The version D is not proved (for the same reasons, as version A).

1.3. Project prerequisites

Slide *5. Basic_research* mentions some of the studies which form the basis of this project. These are works which prove that nearly all psoriatic patients have SIBO. Hereinafter the term "SIBO" is used to denote the syndrome of small intestine bacterial overgrowth of parietal microbiome on any site (sites) or throughout mucous small intestine.

> For the first time researches of transient microflora of proximal small intestine at 121 psoriatics (PASI >=20) are conducted by Natalia Potaturkina-Nesterova with co-workers (2007-11). There are 52 people with moderate psoriasis (PASI in range 20-30) and 69 people with severe psoriasis (PASI more than 30). At all patients psoriasis was in progressing stage. 43 healthy persons have been included into control group. Level SIBO more than 10^5 CFU/ml (TBC > 5) was found at 95 (78.5%) psoriatics.
> TBC for psoriatic patients has made - on average $3x10^6$ CFU/ml (lg=6.49) that is much more than in the control group – on average $1.1x10^3$ CFU/ml (lg=3.05). Correlation between SIBO level and PASI (r = 0.46), between SIBO level and duration of psoriasis disease (r = 0.43) has been found. (Gumayunova 2009a, Gumayunova 2009b, Peslyak 2012d),.
>
> At 93% of psoriatic patients Bifidobacterium spp. was found - on average $2x10^5$ CFU/ml (lg=5.3). In control group at 40%, on average 250 CFU/ml (lg=2.41). At 84% of psoriatic patients Lactobacillus spp. was found - on average $4.6x10^4$ CFU/ml (lg=4.66). In control group at 19%, on average 350 CFU/ml (lg=2.54). At 79 of 121 psoriatic patients (65%) Enterococcus spp. was found - on average $2x10^5$ CFU/ml (lg=5.28). Enterococcus spp. are not found in control group at all. At part of psoriatic patients Str.pyogenes (9%) and Str.viridans (30%) were found (not found in control group) (slides *8. SIBO-1 and 9. SIBO-2).*
>
> According to the results of cultural proximal SIBO-tests of PP one or more species presumed psoriagenic are found in small intestine microbiome taken in the zone of Treitz ligament. If two (or more) biomaterials were investigated, then the maximum value is specified. Most of the examinations were carried out in the Federal State Budgetary Institution “N.I. Pirogov National Medical and Surgical Center” (slide 10. SIBO_Moscow).

Fragments of bacterial products of small intestine bacteria contain PAMP: lipopolysaccharide (LPS) and peptidoglycan (PG), including specific peptidoglycan (PG-Y). With increased macromolecular small intestine permeability (Korotky 2005, Harkov 2005, Harkov 2006, Harkov 2008, Ely 2018, slides *6. Permeability-1* and *7. Permeability-2*) in psoriatic patients, these bacterial products get to systemic blood flow, form the chronically increased PAMP level in blood and chronically increased PAMP-load on blood phagocytes. Specifically, they form the increased LPS-level (Garaeva 2007).

Phagocytes are neutrophils, monocytes and dendritic cells (Nagl 2002).

The specific peptidoglycan PG-Y contains Y-antigen, i.e. peptide which includes interpeptide bridges (L-Ala)-(L-Ala) and/or (L-Ser)-(L-Ala). Such bridges are found in nearly all species of Streptococcus, in Enterococcus faecalis, and also in many species from Leuconostoc and Weissella genera (slides *16. PG_PsB-1* and *18. PG_PsB-3*). Such species are presumed psoriagenic (Baker 2006a, Baker 2006b, Peslyak 2012a, Peslyak 2012b, Ely 2018), hereinafter designated PsB (section 5.4).

Slide *5. Basic_research* also mentions studies of PAMP-load influence on phagocytes (Adib-Conquy 2009, Biswas 2009). From these (and other similar studies) it is known that PAMP-load (especially synergic PG- and LPS-load) affects blood phagocytes by activating them. If this occurs, part of activated monocytes and dendritic cells is tolerized. In tolerized monocytes Mo-T and dendritic cells DC-T the level of proteins responsible for endocyted bacterial products degradation is reduced. Thereof tolerized Mo-T and DC-T can become carriers of non-degraded bacterial products, particularly Y-antigens.

The total of the given subprocesses, leading to the emergence of tolerized Mo-T and DC-T - carriers of specific antigen material in systemic blood flow, within Y-model of pathogenesis is called SPP - systemic psoriatic process. Tolerized Mo-T and DC-T get a chemostatus similar to the non-activated one and can therefore be attracted to places of inflammation in any tissue, particularly into derma. Being attracted into inflamed derma, tolerized Mo-T and DC-T affected by cytokines are reprogrammed and transform into mature dendritic cells. Some of them (monocytes Mo-R and dendritic cells DC-R) contain Y-antigens. Mature dendritic cells (formed from Mo-R and DC-R) present Y-antigens to specific T-lymphocytes (slide *24. Local_processes_Y*). The systemic Y-model of pathogenesis was first formulated in the monograph (Peslyak 2012a, Peslyak 2012b).

After the publication of Y-model there appeared results of new researches, primarily connected with netosis of neutrophils, including that associated with psoriatic disease. It became known that a considerable proportion of blood neutrophils in psoriatic patients is in the activated (prenetotic) state (Hu 2016, slides *62. Net-blood-1*, *63. Net-blood-2*, *64. Net-blood-3*).

Within new YN-model such a condition of blood neutrophils is also dictated by chronically increased PAMP-load (section 5.2).

Getting into inflamed psoriatic skin, many blood neutrophils undergo netosis (Lin 2011, Hu 2016, slides *61. Netosis*, *65. Net-skin-1*, *66. Net-skin-2*). As a result, in the intercellular space there can appear non-degraded bacterial products which were earlier endocyted by neutrophils in blood, including Y-antigens (slide *25. Local_processes_YN*). Bacterial products lost during netosis are endocyted by all skin phagocytes. Monocytes Mo and dendritic cells DC which endocyte non-degraded fragments of PG-Y become Y-antigen

carriers (hereinafter Mo-Y and DC-Y). Under the influence of pro-inflammatory cytokines some monocytes Mo-Y are transformed to dendritic cells MoDC-Y.

In pro-inflammatory environment MoDC-Y and DC-Y continue transformation and transform to mature dendritic cells maDC-Y. These maDC-Y present Y-antigen to effector TL-Y (Y-specific T-lymphocytes) (subprocess LP8.1, slides *25. Local_processes_YN* and *26. YN-model*).

This presentation initiates and supports the false acquired answer to imaginary skin PsB-infection, one of its consequences being hyperproliferation of keratinocytes (subprocess LP8.2).

Subprocess LP8 fully coincides within YN-model and Y-model.

The transfer of Y-antigen in psoriatic skin from neutrophils to monocytes and dendritic cells due to netosis is the main difference between YN-model and Y-model (slide *25. Local_processes_YN*).

A detailed description of all the differences between YN-model and Y-model can be found in section 5.2.

Within the project it is proposed to receive new facts in favour of several basic hypotheses underlying systemic Y-model and YN-model (Table 2, slide *27. NCS1_Hypo*). The full list of hypotheses for Y-model was formulated earlier (slides 42 and 43, Peslyak 2012c). The comparison of all hypotheses for Y-model and YN-model is made in Supplement S6, section 5.12).

Table 2. Hypotheses tested within the project.

	Hypothesis	**Test**
H1-1	One of the two main reasons of systemic psoriatic process SPPN (as well as SPP) is increased macromolecular small intestine permeability (including that for bacterial products). (slides *6. Permeability-1* and *7. Permeability-2*)	Direct
H2	PsB have PG-Y - peptidoglycan with interpeptide bridges IB-Y, i.e. (L-Ala)-(L-Ala) and/or (L-Ser)-(L-Ala). Y-antigen is a part (or parts) of interpeptide bridge IB-Y. (slides *16. PG_PsB-1* and *18. PG_PsB-3*)	Indirect
HN3	PAMP-nemia and (PG-Y)-nemia are the main subprocesses. kPAMP are LPS, PG and bacDNA. (slides *22. SPPN-PAMP-nemia* and *23. SPPN*)	Direct
HN10	The attraction of (PG-Y)+ phagocytes to skin from blood flow is an essential link of the vicious cycle. The existence and severity of any psoriatic plaque is determined by the intensity of Y-antigen income, carried by these blood phagocytes. For more detail see section 5.2.	Indirect. HN10-S will be actually tested.
HN10-S	Non-degraded non-host biomaterial is transferred to psoriatic skin in blood phagocytes (slide *29. HN10-S*)	Direct

The main objective of the project is testing and, hopefully, confirmation of these hypotheses (direct or indirect).

To achieve this, a new approach developed and implemented at the Institute of Cardiovascular and Metabolic Diseases, Toulouse, France (2011-2016) will be taken (slides *5. Basic_research* and *34. Blood-bacDNA (France))*. This group of researchers first identified whole blood metagenome (16S-test) in a big group of healthy persons (Paisse 2016). Such researches (including those using WMS-tests) are conducted with ever increasing frequency for various diseases and make it possible to elicit previously unknown facts about the role of microbiome in their pathogenesis (sections 2.1 and 2.2).

In this project whole blood metagenomes of psoriatic patients and of the control group will be identified and studied for the first time. The concentration of nhDNA (non-host DNA) for separate species and the total by subgroups will be determined. Macromolecular small intestine permeability will be determined by new bacDNA-test, and their correlations with PD severity will be elicited (testing hypotheses H1-1, H2 and HN3). Metagenomes of psoriatic skin biopsies (or only its phagocytes: the decision will be made following the results of the pilot stage) will also be determined, followed by their complex study together with whole blood metagenomes (sections 2.3 and 2.5). This is to prove the income of non-degraded bacterial products to psoriatic skin in blood phagocytes (testing hypotheses HN10 and HN10-S).

2. Metagenomic sequencing

2.1. Blood metagenome

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 3).

bacDNA concentration in plasma is more than 3 orders lower than in buffy coat: $1.5*10^4$ (16S copies)/ml against $4.2*10^7$ (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 endocyted (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. 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 (fig.2, Okubo 2002, slide *31. Okubo*).

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 (slide *32. Blood_Psor*). 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 (section 5.12).

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 and 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 (slide *33. Blood_WMS_and_Culture*). 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 (slide *34. Blood-bacDNA (France)*) 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 like Proteobacteria (87%) and Bacteroidetes (classes Sphingobacteriia, Bacteroidia and Flavobacteriia) (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 – $1.4*10^4$ (16S copies)/ml on average – is of the same order with bacDNA concentration found in the control test of one of the reagents – $1.5*10^4$ (16S copies)/ml.

The results for whole blood have sufficient reliability as bacDNA concentration amounted from $1.8*10^7$ to $7.6*10^7$(16S copies)/ml). On average $4.2*10^7$ (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 (Supplemental Fig. 2a, Païssé 2016). 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 results of this research to within genus are given only for the main pathogens: Acinetobacter ~ $6*10^5$ (16S copies)/ml; Corynebacterium ~ $4*10^6$ (16S copies)/ml, Escherichia or Shigella ~ $1.5*10^5$ (16S copies)/ml (16S-test does not distinguish between these two genera), Pseudomonas ~ $1.5*10^6$ (16S copies)/ml, Staphylococcus ~ $1.7*10^5$ (16S copies)/ml, Stenotrophomonas ~ $2.3*10^5$ (16S copies)/ml. 16S average concentration found in buffy coat is specified (at the rate on 1 ml of whole blood). Genus of bacteria Shewanella ~ 10^4 (16S copies)/ml is found only in plasma. (Païssé 2016, fig. S3).

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 (Lluch 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 $2.4*10^3$ (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) (slide *35. Blood-Germany-1* – for some patients). 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.

This enabled us (with the use of results received by the authors for 15 HP – only for them the data have been published) to estimate the total representation of species presumed psoriagenic (~5.7%). In this case the probable skin contaminant P.acnes was excluded (similar to Horiba 2018) (slide *36. Blood-Germany-2*).

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 (for more detail about this and similar kits see – section 5.7). 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 (section 5.7).

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% (Suppl. fig.3A, Puri 2018).

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 $1.38*10^8$ (16S copies)/ml - slide *37. Blood-bacDNA (China)*. 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.

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. Precisely this approach will be particularly efficient for our project.

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 (correct Tab.2, Qian 2018). which corresponds to averagely $1.17*10^8$ (16S copies)/(ml of whole blood).

Table 3 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 (Païssé 2016, Puri 2018, Li 2018, Qian 2018). 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.).

While this project version was being prepared, it was decided to abandon preliminary isolation of phagocytes from whole blood and to carry out isolation of all DNA from whole blood at once with the help of one of standard kits (section 5.10, par.6). Firstly, such an approach will minimize the time between blood sampling and destruction of blood phagocytes, which will make it possible to preserve the maximum amount of nhDNA; secondly, phagocytes constitute 60-70% of all blood leukocytes and therefore abandoning their isolation will insignificantly increase hDNA proportion; thirdly, refusal of performing isolation of blood phagocytes (long procedure similar to *77. Phagocytes selection*) will reduce the probability of contamination.

The results can be also affected by contamination (of one or several reagents, casual, etc.). For more detail about contamination see (Glassing 2016), which is entirely devoted to this problem and contains reasonable criticism of some of the above listed works. Section 5.7 contains the description of how it is supposed to control contamination level in this project.

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 3). Part of the same information is given in another format (Table 4) demonstrating project novelty.

The procedure of preparing patients and implementing WMS-test of whole blood, search of correlations with PASI is nuanced in 5.10.

Table 3. Blood metagenome researches. Report.

Patients	Biomaterial	Test	Determining concen-tration	Contami-nation control	nhDNA (particularly bacDNA)	Study (year, country), notes, reference to bioproject.
PP (psoriatic patients) and HP						
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	Mono-cytes	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 2010 (UK). Metagenome was not identified. Slide *32. Blood_Psor*.
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	Leuko-cytes	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	Leuko-cytes	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. Slide *33. Blood_WMS_and_Culture*.
30 HP	Whole blood and 3 fractions	16S	yes	yes, NTC, 0.05%	bacDNA in all	Païssé 2016 (France), HP - donors (postprandial blood tests). Increased bacDNA concentration provided by (Lluch 2015) method. Slide *34. Blood-bacDNA (France)*. 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 Slides *35. Blood-Germany-1* and *36. Blood-Germany-2*

Patients	Biomaterial	Test	Determining concen-tration	Contami-nation control	nhDNA (particularly bacDNA)	Study (year, country), notes, reference to bioproject.
9 patients	Non-host cells in whole blood	WMS	no	yes	nhDNA in all	Gyarmati 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).
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.
56 patients, 20 HP	Whole blood	16S	yes		bacDNA in all	Puri 2018 (USA), patients with alcoholic syndrome, DNA isolation procedure is similar to Païssé 2016.
50 patients and 12 HP	Whole blood, neutro-phils	16S	yes	yes	bacDNA in all	Li 2018 (China), patients with pancreatitis, slide *37. Blood-bacDNA (China)*
45 HP and 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.
NCS1 research project						
15 PP, 5 HP	Whole blood	WMS	yes	yes	nhDNA in all	(2019-20, 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 4. 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 (+)		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, +)	**NCS1 (+)**	**NCS1 (+)**

Note: + - concentration was (will be) determined.

As the project presupposes determining concentration of bacDNA and nhDNA in whole blood, the following section is devoted to this question.

2.2. bacDNA concentration in HP 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 5). All these studies have been described above (section 2.1, Table 3).

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. For more detail on efficiency of NME use see section 5.7.

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.

There are 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 tasks T1.1 and T1.2 (pilot stage) 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). In our project after sampling postprandial blood, preprocessing by RLT buffer is to be performed as soon as possible.

Table 5. bacDNA concentration in HP blood. Report.

Study	Okubo 2002	James 2011	Païssé 2016	Puri 2018	Li 2018	Qian 2018
Country	Japan	Canada	France	USA	China	China
Biomaterial	Monocytes	Leukocytic mass	Whole blood (and other fractions)	Whole blood	Whole blood	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)
Time of blood sampling	ND	ND	after drink and meal	ND	after fasting	without control
Preprocessing of samples before DNA isolation			Homo-genization	Homo--genization	RLT buffer	Phenol / chloroform
Whole blood. (16S copies)/(ml of whole blood)		4.29*E7 (recalcula-tion)	4.20*E7	1.65*E6 (recalcula-tion)	1.38*E8	1.17*E8 (recalcula-tion)
Determination of bacDNA concentration (qPCR)	-	V3, special primers, 194 - amplicon	V3-V4, EUBF, EUBR, 467 - amplicon	V3-V4, universal primers, ND	V3, 357f/518r, 162 - amplicon	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%
(16S copies)/monocyte (Okubo 2002) (16S copies)/leukocyte (Paisse 2016) (recalculation)	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	?
Contamination control			NTC		NTC >=5	NTC=6
Contamination level			0.048% (average)		0.003% (maximum)	0.138% (average)

Besides determining bacDNA concentration by the quantity of 16S copies, it will be also possible to determine concentration of any mapped species by WMS-test results, and, consequently, to determine the total concentration of any subsets of mapped species (Supplement S2, section 5.12). And it will become possible to perform comparison of total bacDNA concentration received by these two different methods. Biochemical tests for determining concentration of lipopolysaccharide and peptidoglycan in blood will be also carried out (section 5.6).

2.3. Skin metagenome

Microbiome of normal and psoriatic skin has frequently been studied and compared. It is well known that Staphylococcus aureus, Malassezia Species, Candida Albicans in psoriatic plaques are found more often than normal, which, as a rule, aggravates inflammatory process (Alekseyenko 2013, Fry 2016, Fry 2013, Gao 2008, Fomina 2009). It is also known that infection of PP healthy skin which affects derma can cause psoriatic plaque initiation (Koebner effect). In certain cases eliminating infection from psoriatic plaque can lead to its subsidence and even to disappearance. However, complete disappearance hardly ever takes place. None of skin bacterial pathogens is regarded as the main reason for initiation and support

of psoriatic plaques. All of them are viewed as triggers (provokers of initiation) and/or as factors aggravating severity of psoriatic plaque.

Metagenome of normal skin as well as of skin with various diseases has been actively studied in the last 10 years. In most studies, smears or scrapes were used as biomaterials, biopsies were studied only in two works (Fahlen 2012, Nakatsuji 2013). WMS-tests were applied only to smears or scrapes.

In (Gao 2008) the presence of bacDNA in skin smears from HP and PP was investigated. Smears from PP were taken from apparently healthy skin and from psoriatic plaques. Analytical processing of 16S-test results was applied, permitting to interpret OTU to within species (SLOTU method). 19 smears were taken from 6 PP (13 from psoriatic plaques and 6 from apparently healthy skin). All in all 1925 clones were detected (2038 - in HP). Representation of Propionibacterium sp. was lower on psoriatic plaques (~2.9%) than in HP (~21%; $p < 0.001$) and than on PP apparently healthy skin (~12.3%). And vice versa, representation of Streptococcus sp. was higher (~15.2%) on psoriatic plaques ($p < 0.001$) than in HP (~7.1%) and than on PP apparently healthy skin (~3.4%). Among Streptococcus sp. found in PP, the highest percentage belonged to Str.mitis (~5.6%) and Str.salivarius (~2.3%). Str.pyogenes was not detected at all. Concentration was not determined. The authors presumed that differences in skin microbiome might be related to PD pathogenesis, but they did not formulate any hypotheses.

In (Fomina 2009) presence of both HPV (PCR test) and bacterial microbiome (culture) in PP smears was investigated. DNA HPV was found in 87% of PP samples and only in 44% of HP samples ($p < 0.001$). The virus load HPVL = lg((number DNA HPV)/(10^5 human cells)) was determined. It turned out that HPVL < 2 for 40% of HPV+PP samples and for 83% of HPV+HP samples, 2 < HPVL < 3 for 45% of HPV+PP samples and for 17% of HPV+HP, and HPVL > 3 for 15% of HPV+PP samples and for 0% of HPV+HP. I.e. HPVL for PP proved to be definitely higher than for HP. It was also demonstrated that HPVL is definitely lower for PP in remission stage compared to aggravation stage. HPVL increase in psoriatic skin correlates with disturbances of microbiome: specifically, S.aureus quantity increases considerably.

In (Fahlen 2012) 16S-test (variable V3-V4 areas) was first used to research skin biopsies. These (2 mm diameter) were taken from 10 PP and 12 HP. PP had not received topical treatment for two weeks, and ultraviolet or systemic treatment for a month before that. The results were sorted into 19 phylums, 265 taxons and 652 OTU with 97% identity. Three most common phylums were Firmicutes (39% in PP, 43% in HP), Proteobacteria (38% in PP, 27% in HP) and Actinobacteria (5% in PP, 16% in HP Streptococcus sp. proved to be well represented in PP (32% - found in all biopsies) and in HP (26%). Staphylococcus sp. was less represented in PP (5%) than in HP (16%) (slide *38. Skin-bacDNA*).

Two studies (Alekseyenko 2013, Statnikov 2013) contain detailed analysis of 16S-test results of smears from a large group of PP (n=54) and HP (n=112). All samples could be subdivided into two clusters representing slightly different microbial communities. Metagenome of psoriatic plaque smears was mainly found in Firmicutes and Actinobacteria cluster whereas metagenome of HP skin smears – in Proteobacteria cluster. When a few months later (during which treatment of psoriasis was carried out) smears from the same plaques of certain PP were retested, membership of the same cluster was retained.

It was discovered that total representation of Corynebacterium, Staphylococcus and Streptococcus genera in psoriatic samples was increased whereas, on the contrary, representation of genera Cupriavidus, Flavisolibacter, Methylobacterium and Schlegelella was reduced (compared to HP samples). It is of interest that representation of Acidobacteria Gp4 order positively correlated with PASI. The authors did not draw any conclusions on the connection between the discovered correlations and PD pathogenesis.

In (Jagielski 2014) only fungi presence in smears taken from 6 PP, 6 HP and 6 AD patients (with atopic dermatitis) was investigated. Malassezia sympodialis proved to be the prevailing species (82.9%) found in the process of cultivating 29 samples. Only M.sympodialis species were found in AD patients while M.furfur species were discovered only in PP. M.sympodialis was more frequently found in AD patients and HP than among PP. Concordance between phenotypical and molecular methods was high (65%). All Malassezia species were susceptible to cyclopiroxolamine and azole, whereas M.furfur species were tolerant to a larger number of medicines than others.

In (Takemoto 2015) only fungi presence in 12 PP and 12 HP was investigated (samples were taken by tweezers or on special bandage). 317806 qualitative sequences corresponding to 142 fungi genera were received. A larger variety of genera but a lower representation of Malassezia (46.9%) was found in PP compared to HP (76%). Malassezia was the richest phylum for both PP and HP. The proportion of Malassezia globosa fractions to Malassezia restricta was lower in PP than in HP. In this research, as well as in the previous ones, no correlation between the representation of any fungi genera and PASI was discovered.

In dissertation (Tanes 2015) the results received for PP within Human Microbiome Project (Supplement S7, section 5.12) are analyzed. On the basis of 16S-test results for 155 smears by means of QIIME and PICRUST major genes of PP microbiome were studied (their inventory and quantity were determined algorithmically). Significant changes in host genes were also studied; search of interrelations was carried out. Not without interest is the approach in this study, where gene structure of microbiome is simulated and studied on the basis of limited information, which 16S-test gives.

Detailed reviews of the above-mentioned and some other (earlier and less significant) researches of skin microbiome at psoriasis are made in two recent works (Fry 2016, Yan 2017).

Now let us consider several works in which HP skin microbiome was studied and/or skin microbiome at other diseases (besides psoriasis). At that, either biopsies were studied or WMS-testing was applied.

In (Nakatsuji 2013) bacDNA presence in skin biopsies (n=11) was investigated. Biopsies were obtained as follows. Post-surgical healthy skin was cleared by sterile scalpel and subsequently sterilized by tampon. After that, by means of 6 mm punch, biopsy intake of 2-3 mm depth was carried out so that epidermis, derma and part of adipose tissue located under derma got into it.

Then biopsy in sterile conditions was divided into 30-50 micron layers which later went through 16S-test separately. The results were grouped into four sections: epidermis, follicular derma, derma and adipose tissue. bacDNA proved to be present not only in epidermis and follicular derma, but also in underlying layers of derma (which do not contain follicles or sweat glands) and in adipose tissue (slide *39. Derm-16S-1*).

bacDNA representation to within order is specified in the table (supplements to Nakatsuji 2013) and to within class – in the chart (slide *40. Derm-16S-2*). Determining absolute quantity (concentration) was made for several characteristic bacteria (Propionibacterium acnes, Staph. epidermidis and Pseudomonas sp.) in additional 16S-tests with specific primers. Pseudomonas genus bacteria (from Pseudomonadaceae family) were found in follicular derma - 4200 CFU/mm^3 on average, and in non-follicular derma - 500 CFU/mm^3 on average.

According to 16S-test results (Fahlen 2012) for HP control group (n=13), bacteria of Pseudomonadaceae family constitute 2% of the total amount found (slide *38. Skin-bacDNA*). Comparing results of (Fahlen 2012) and (Nakatsuji 2013) for this family enables us to estimate the range of normal dermal bacDNA concentration from 500/0.02 to 4200/0.02 CFU/mm^3, i.e. from 2.5*10^4 to 2.1*10^5 CFU/mm^3.

At present there are no other papers studying bacDNA presence in skin biopsies by methods of metagenomic sequencing (Ferretti 2017).

In (Bouslimani 2015) by 16S-test for two HP biogeography of skin microbiome distribution was first studied (smears from 400 sites for each HP). Slide *42. Skin_Bacteria_3D* shows 3D distribution for Streptococcus and Staphylococcus genera.

In (Oh 2014) systematic WMS-testing of smears from human skin taken from various sites was first made (15 HP, 18 sites with various microenvironments – dry, damp, greasy, toe nail, etc. - slides *43. Skin-WMS-18-1* and *44. Skin-WMS-18-2*). Mapping on the following reference DB was carried out: National Center for Biological Information (NCBI), Human Microbiome Project (HMP), Saccharomyces Genome Database (SGD), Fungal Genome Initiative (FGI), FungiDB; nonreference method was also applied. Microorganism communities and specifically DNA viruses, fungi, bacteria, including subspecies and strains of the dominant bacteria, were determined. It was demonstrated how functional capacities of microorganism community depend on localization of site on the body, a multidomain catalog of skin microorganism genes was created. Clusters of species which do not have reference were identified. It was demonstrated that biogeography and the individual determine functional and taxonomical characteristics of microorganism communities to a great extent. All in all 263 smears (9 men, 6 women) were studied. The amount of host DNA constituted from 19.4% (internal epithelium of nostril) to 98.2% (heel) of reads. The quality of reads and effective coverage depended on sites (from 38% to 81%). All in all 289 Gbp (giga base pairs) of filtered reads of nhDNA were received and analyzed. The basic data and final results are published and freely available in Excel format.

In (Hannigan 2015) comparative WMS-testing of virome and whole metagenome of skin was first carried out (16 HP, smears from 8 contralateral sites). To study virome, biomaterial was first purified of everything, except for virus particles, out of which DNA was subsequently isolated (optimized enrichment method). Mapping was carried out with the use of native and existing software. It was shown that both virome, and whole metagenome strongly depend on microenvironment (i.e. from particular skin sites). In virome genes of moderate phages were mainly found. Spacers CRISPR found in bacterial genomes partly made it possible to prove coexistence of phages and bacteria in communities (as a rule, rich in Corynebacterium sp.) as well as biogeography of phages. Such detailed study of skin virome had been

impossible because of low concentration of virus DNA in and on skin. However, modern methods of sequencing which work with ultra-small DNA quantities (<1 ng), made this research possible.

Virus DNA was also found in whole metagenome of skin (0.4% of all reads). Part of virome reads (17% on average) were found in whole metagenome. In virome, the greatest part was constituted by phages, but human papillomaviruses (HPV), notably with maximum representation on HP palms, were also discovered.

In (Chng 2016) 39 people were examined (19 AtD+ - patients with of atopic dermatitis record, 15 HP with SPT(-) - negative skin prick test and 5 HP with SPT+ - positive skin prick test). Two smears from each patient (from right and left elbow folds – antecubital fossae) with the use of D-Squame Standard Sampling Discs (tape-stripping method) were taken. The results of two WMS-tests were averaged. For several patients extra 16S-test was carried out, which made it possible to compare its results with WMS-test results and to demonstrate the advantages of WMS-test.

The authors singled out seven genera and nine species which manifested the most significant differences among the surveyed groups. Slide *46. Skin-AtD-WMS-genus* shows the results for genera of Streptococcus and Gemella (increased representation compared to control) and Dermacoccus (reduced representation compared to control). Among the nine species, there are three species of alpha-hemolitic streptococci. Most species. whose representation is increased in AtD+ patients, are commensals or conditional pathogens of URT.

Species representation (for Streptococcus and Staphylococcus genera) was studied (slides *47. Skin-AtD-WMS-Strep*, *48. Skin-AtD-WMS-Staph*). And for pathogenic species Staphylococcus aureus – strain representation was studied (slide *49. Skin-AtD-WGS-Strain*). The authors specified that Str. pyogenes was found in none of the surveyed. Such reliable species specification and any strain specification is only possible with WMS-test results. At the moment, it is the first and only work, where WMS-test was carried out for dermatological patients, which makes it important for the project. The basic data and final results of this work are freely available on NCBI. Sequence Read Archive and on Nature Microbiology magazine website.

In (Tett 2017) 28 PP were examined. 48 smears of psoriatic plaques and 49 smears of apparently healthy skin (97 smears in total) were taken. Sites which are most characteristic for plaques, such as RC – retroauricular crease and OS – olecranon skin area (right and left), were chosen for smears. Such PP were selected, at least one of whose sites was without plaques. After studying WMS-test results it became clear that for 48 smears taken from plaques only 4.9% reads on average were mapped on reference DB, while for smears from apparently healthy skin the number of such reads averaged 23.1%. The main reason for it was a higher percentage of hDNA in smears from affected sites.

Irrespective of psoriatic plaque presence or absence, by means of taxonomical analysis (MetaPhlAn 2) it was demonstrated that skin metagenomes mainly consist of Actinobacteria and Firmicutes phylums. The following species have the highest representation: Staphylococcus epidermidis, Propionibacterium acnes, Staphylococcus caprae/capitis and Micrococcus luteus as well as fungi of Malassezia genus (slide *50. Psorskin-swab-WMS-1*).

Representation of Staph.epidermidis and P.acnes from PP skin smears are similar to those received in other studies of HP skin metagenomes. The authors emphasize that conclusions in those previous studies coincide with their own conclusion that there is no specific essential difference between psoriatic skin metagenomes and HP skin metagenomes. Moreover, for the first time it was demonstrated by means of WMS-tests, i.e. at species level.

To investigate intraspecies distribution, new strain-specific StrainPhlAn and MetaMLST methods were applied. This made it possible to construct two wholegenomic phylogenetic trees for Staph.epidermidis and P.acnes strains from samples with sufficient coverage (> 2) (slide *51. Psorskin-swab-WMS-2*). Cartography of ear samples from three patients demonstrates similarity of strain composition for right and left ear without plaques (patients P4 and P9) and difference of strain composition for left ear with psoriatic plaques and right ear without them (patient P16). Points in the figures correspond to contigs which have a color determined by species. The arrangement of contigs is determined by GC content and representation, the black color indicates unknown taxon, RC and OS denote ear and elbow samples respectively.

In (Loesche 2018) smears from psoriatic plaques and apparently healthy skin in 114 PP were studied by 16S-test. Smears were taken from 6 various localizations (arm, axilla, buttock, leg, scalp, trunk) triply (week 0, 4 and 28) during long-term ustekinumab treatment. Metagenomes of psoriatic and apparently healthy skin of the same localization are similar in their composition and representations (slide *52. Psorskin-6*). Besides, no fundamental differences between metagenomes identified at different stages of ustekinumab treatment were found.

Some reviews deserve attention, as they analyze the most interesting studies and give a comparative characteristic of their research methods. Results of researching (16S-test) smears taken from

4 HP in 20 various skin sites are given in (Grice 2011). The authors emphasize that WMS-test will be able to give more information, if preliminary hDNA elimination is carried out. By now several methods of hDNA elimination (section 5.8) have been developed. There are published results of WMS-tests of skin (Chng 2016, Ferretti 2017, Oh 2014, Hannigan 2015) in which preliminary hDNA elimination is applied in a varying degree. In (Grice 2011) reviews research results on the presence of viruses, fungi and even mites on skin and in skin.

Slide *41. Healthy-skin* (see left) shows a scheme of microorganism presence in healthy skin, reflecting the ideas existing at the time of review preparation (Grice 2011). The scheme on the right (the same slide) reflects the understanding of microorganism presence (or rather their bacDNA) at the moment (including results of Nakatsuji 2013, slide *39. Derm-16S-1*).

The review in (Kornienko 2015) is made by a Russian author and contains a brief result description of skin metagenome researches carried out by foreign authors. It compares the potential of 16S- and WMS-tests and gives characteristics of skin metagenome at various diseases (particularly at psoriasis, by Fahlen 2012 and Statnikov 2013).

The review (Ferretti 2017) contains detailed comparison of 16S- and WMS-test characteristics. It enumerates advantages of WMS-tests, contains the description of advantages and disadvantages of various methods of nhDNA isolation, and assesses works in which preliminary hDNA elimination was carried out. The review discusses contamination control in detail. The author's own research is analyzed (WMS-test of smears from 4 skin sites taken from 3 HP, Truong 2015). It describes the role of MetaPhlAn2 software developed by the authors, which makes it possible to interpret WMS-test results to within strain level. Some of the authors of this review are staff members of SegataLab laboratory which has developed software with a similar potential.

The review (Fry 2016) is to a greater extent a historical one as it describes in detail all researches of psoriatic skin metagenome starting with the earliest ones.

The review (Yan 2017) is devoted to PD metagenome study. It analyzes in detail many of the above listed works, and it points out that psoriatic skin virome has not been identified yet. Besides, a whole section is devoted to results of studying fecal metagenome at PD.

The review (Kong 2017) contains detailed information about planning any experiment on determining and researching skin metagenome. Recommendations given in this review must undoubtedly be considered in the detailed preparation of the project.

The review (Niemeyer 2018) contains the most detailed list of the skin metagenome researches carried out for the following diseases: atopic dermatitis, acne, psoriasis, hydradenitis, seborrheic dermatitis and trophic ulcer.

The works listed above (containing results of the authors' own researches) are summarized in the report (Table 6). Next, part of the same information is given in another format (Table 7), which highlights project novelty.

Table 6. Skin metagenome researches. Report.

Patients	Biomaterial	Test	Determining concentration	Contamination control	nhDNA (particularly bacDNA)	Study (year, country), notes, reference to bioproject.
PP (psoriatic patients) and HP						
6 PP, 6 HP	smear (19,20)	16S	no		bacDNA	Gao 2008 (USA), see Streptococcus sp.
69 PP, 46 HP	smear	cult, PCR	yes		PCR for HPV	Fomina 2009 (Russia)
10 PP, 12 HP	biopsy	16S	no	yes	bacDNA	Fahlen 2012 (UK), Slide *38. Skin-bacDNA*, see Streptococcus sp.
54 PP, 112 HP	smear	16S	no		bacDNA	Statnikov 2013 (USA) with Alekseyenko 2013, see Streptococcus sp.
54 PP, 37 HP	smear	16S	no		bacDNA	Alekseyenko 2013 (USA) dbGaP: phs000251 Genbank: PRJNA74929 (not mentioned in the study) with Statnikov 2013, see Streptococcus sp.
6 PP, 6 HP, 6 AD	smear (29)	cult, PCR	no		DNA of fungi	Jagielski 2014 (Poland)
12 PP, 12 HP	tweezers bandage	26S	yes		DNA of fungi	Takemoto 2015 (Japan)
?	smear (155)	16S	no	-	bacDNA	Tanes 2015 (USA). On HMP results.

Patients	Biomaterial	Test	Determining concentration	Contamination control	nhDNA (particularly bacDNA)	Study (year, country), notes, reference to bioproject.
28 PP	Smear (97)	WMS	no	-	nhDNA	Tett 2017 (Italy), strain studying Genbank: PRJNA281366, Slides *50. Psorskin-swab-WMS-1* and *51. Psorskin-swab-WMS-2*
114 PP	Smear (114x6x3)	16S	no	-	bacDNA	Loesche 2018 (USA), 6 sites during ustekinumab treatment (week 0,4 and 28). Slide *52. Psorskin-6*
Non-psoriatic patients and/or HP						
11 patients	biopsy (> 6x4)	16S	yes	yes	bacDNA	Nakatsuji 2013 (Japan), first proved dermal presence of bacteria. Genbank: PRJDB716. Slides *39. Derm-16S-1*, *40. Derm-16S-2*.
15 HP	smear-scraping (263)	WMS, 16S	no	yes	nhDNA (bacteria, fungi, viruses, archaea)	Oh 2014 (USA), integrated reference catalog > 4000 species, mapping to strain level for two species, 16S - for control. dbGAP: phs000266 Genbank: PRJNA46333 Slides *43. Skin-WMS-18-1*, *44. Skin-WMS-18-2* and *45. Skin-WMS-18-3*.
2 HP	smear (2x400)	16S	no	yes	bacDNA	Bouslimani 2015 (USA, Germany), 3D biogeography. Slide *42. Skin_Bacteria_3D*, see Streptococcus sp.
16 HP	smear (16x2x8)	WMS	no	yes	separately virus DNA, separately all nhDNA (bacteria, fungi, viruses)	Hannigan 2015 (USA) Genbank: PRJNA266117
19 AtD+ patients, 15 SPT(-) HP, 5 SRT+ HP	sticky disc, washout (> 78)	WMS, 16S	no	yes	nhDNA (bacteria, fungi, viruses)	Chng 2016 (Singapore), 16S – only for comparison on bacDNA for several patients, mapping on strain level for Staph.aureus Genbank: PRJNA277905 Slides *46. Skin-AtD-WMS-genus*, *47. Skin-AtD-WMS-Strep*, *48. Skin-AtD-WMS-Staph* and *49. Skin-AtD-WGS-Strain*, see Streptococcus sp.
Reviews including the authors' own results						
10 HP	smear (10x20)	16S	no	no	bacDNA	Grice 2011 (USA), review
3 HP	Smear (3x4)	WMS	no	no	nhDNA	Ferretti 2017 (Italy), review and Truong 2015 (Italy), mapping on strain level
NCS1 research project						
15 PP	biopsy of psoriatic skin	WMS	yes	yes	nhDNA	(2019-20, Russia), preliminary elimination of hDNA. According to pilot stage results, a decision on DNA isolation from biopsy or from previously isolated phagocytes will be made.

Table 7. Skin metagenome researches. Fact and plan.

	16S-test (PP and HP)	16S-test (for HP only)	WMS-test (PP)	WMS-test (HP)
Smear, scraping	Gao 2008; Statnikov 2013; Takemoto 2015 (fungi, +)	Oh 2014 Bouslimani 2015 (3D); Chng 2016	Tett 2017; Loesche 2018	Oh 2014, Hannigan 2015; Chng 2016
Biopsy	Fahlen 2012	Nakatsuji 2013 (for the first time, +)	**NCS1 (+)**	

Note: + - concentration was (will be) determined.

Some works demonstrate increased prevalence of Streptococcus sp. on skin (mainly species commensal for URT) (Gao 2008, Fahlen 2012, Alekseyenko 2013, Statnikov 2013, Bouslimani 2015, Chng 2016). Slides *42. Skin_Bacteria_3D* and *44. Skin-WMS-18-2* shows clearly that such prevalence in HP occurs on face and hand skin. The fact is quite predictable as any person regularly touches their mouth (consciously and involuntarily) with their fingers or back of hand. Some people also moisten their fingertips with saliva (e.g. when turning pages), in which case saliva (containing bacteria commensal for URT) gets on fingers and hands and through them – on their face and other body parts.

It should be noted that increased prevalence of Streptococcus sp. in PP occurs on all psoriatic plaques irrespective of their site. It is also easily explained as nearly every psoriatic plaque is subject to regular finger touching (conscious and involuntary wetting by saliva, scratching, peeling, rubbing in of gels and ointments, etc.). Exception to this rule is "inconveniently" located plaques, e.g. on the back (especially on its top part) where finger touching is much less frequent. As almost all species from Streptococcus sp. (including URT commensals) are presumed psoriagenic (slide *18. PG_PsB-3*), presence of their bacDNA of non-resident origin in psoriatic biopsy (i.e. brought in phagocytes from blood) is unsurprising. It is necessary to reduce the complexity connected with dividing metagenome of psoriatic skin (phagocytes) into resident (bacDNA from skin Streptococcus sp.) and non-resident subset (bacDNA from blood phagocytes). To achieve this, biopsy should be taken from psoriatic plaques with the smallest probability of resident Streptococcus sp. presence.

2.4. Phagocytes of HP and PP

Slides *54. Skin_2D* and *55. Skin_3D* show spacing of leukocytes (including monocytes and dendritic cells) in healthy skin (Wang 2014b). In psoriatic skin their composition and distribution is essentially different from the norm. Slide *57. Macrophages* shows distribution of CD163+ macrophages (monocytes) (Fuentes-Duculan 2010) and slide *58. Dendritic_Cells* shows distribution of CD11c+ and CD1a+ dendritic cells in healthy and psoriatic skin at moderate-severe psoriasis (Komine 2007, Zaba 2009a).

Slide *56. Phagocytes-1* summarizes information on blood phagocytes and psoriatic skin phagocytes. Quantitative characteristics of blood phagocytes from HP and PP are only slightly different. The main differences are found in psoriatic skin. In healthy skin almost all phagocytes are of resident origin, i.e. they come from precursors of monocytes and dendritic cells – resident dermal stem cells. But in psoriatic plaque the situation is different – up to 80% of phagocytes have non-resident origin, i.e. they are either attracted from bloodstream or derived from cells attracted from blood flow. These are all neutrophils and up to 70% of monocytes-macrophages and dendritic cells. As calculations show, their concentration in the top 0.5-mm skin layer reaches ~41000 cells/mm^3. About 45% of phagocytes are constituted by neutrophils, about 35% - by monocytes (macrophages) and up to 20% - by dendritic cells. The proportion between phagocyte types in psoriatic skin is determined by the increase in average lifetime of macrophages and especially dendritic cells compared to the same values for blood phagocytes (Kabashima 2016, Gaspari 2017).

Appendix (section 5.2) gives a detailed analysis of the role of neutrophils in YN-model of psoriasis pathogenesis. Therefore within the project it is essential to identify and study metagenomes of all phagocytes, not only of monocytes (macrophages) and dendritic cells. Whole blood metagenome (the bulk of which is constituted by phagocyte metagenome) and metagenome of psoriatic skin (phagocytes) will be identified.

2.5. Complex study of whole blood metagenome and metagenome of psoriatic skin (phagocytes)

Y-model and YN-model presuppose that specific bacterial products found in blood phagocytes come to psoriatic skin together with them and support inflammatory process. This assumption is formulated for

Y-model in the form of H10 hypothesis (only for tolerized monocytes and dendritic cells) (Supplement S6, section 5.12) and in the form of expanded HN10 hypothesis for YN-model which includes neutrophils as well (Table 2, slide *27. NCS1_Hypo*, section 5.2).

The project is supposed to prove or refute HN10-S hypothesis which represents an expansion of HN10 hypothesis (Table 2, slides *28. Biotransfer* and *29. HN10-S*). HN10-S hypothesis presupposes that non-degraded non-host biomaterial is transferred to psoriatic skin inside blood phagocytes (within HN10 hypothesis both phagocytes and bacterial products responsible for supporting psoriatic inflammation are nuanced). Proving HN10-S hypothesis will be based on the complex study of whole blood metagenome and metagenome of psoriatic skin (phagocytes). Detecting nhDNA of non-resident origin, i.e. coming to skin in blood phagocytes, in metagenome of psoriatic skin (phagocytes) will be direct proof of HN10-S hypothesis and serious evidence in favour of HN10 hypothesis.

Slide *29. HN10-S* gives a detailed scheme of presumed income of non-host biomaterial (including nhDNA, LPS and PG) to psoriatic skin in blood phagocytes. Psoriatic skin phagocytes (irrespective of their origin) endocyte nhDNA, LPS, PG and other non-host biomaterial of resident origin (i.e. from any microorganisms living on skin and in skin). When this occurs, non-degraded non-host biomaterial earlier endocyted in blood is presumably retained in blood phagocytes. nhDNA of resident and non-resident origin can be differentiated by means of complex studying of whole blood metagenome and metagenome of psoriatic skin (phagocytes). Unfortunately, this cannot be done for another (different from nhDNA) non-host biomaterial. It is expected to determine nhDNA concentration and to estimate concentration of another non-host biomaterial of non-resident origin in psoriatic skin (primarily peptides – presumed Y-antigens) (section 5.5).

The presumed scheme of how whole blood metagenome influences metagenome of psoriatic skin (phagocytes) in dynamics is represented on slide *73. 2Pools-D*, whereas slide *74. 2Pools-S* shows an instant cut for stable psoriatic plaque and suggested formulas describing this influence. Details are given in Supplement S5 (section 5.12).

To discover this influence two independent WMS-tests are carried out. Initial biomaterial for them is whole blood and psoriatic skin (phagocytes) respectively. Psoriatic biopsy in sterile conditions is transformed into cellular suspension from which in immunomagnetic way all phagocytes (neutrophils, monocytes and dendritic cells) (slide *77. Phagocytes selection*) are isolated (an alternative way is isolation of all DNA from biopsy at once). Then maximum hDNA elimination is performed and WMS-test is carried out, at which point reads belonging to hDNA are analytically excluded. Membership of other reads is determined to within species with the use of NCBI RefSeq (reference genomic DB) (Grumaz 2016). For some reads suitable genome cannot be found, but a set of mapped reads is, as a rule, big enough to conduct detailed study of metagenome.

On the basis of processing information on mapped reads, two lists of nhDNA species representation are made up: MB – whole blood metagenome and MPS – metagenome of psoriatic skin (phagocytes).

What is supposed to be done only with the results on MB is described in sections 2.1 and 3.2 (Tasks T2 and T3). Subsequently interpretation and study of results on MPS and MB in complex is discussed.

Simple comparison with MB immediately makes it possible to subdivide MPS into two fractions: R – resident and M – general. If certain nhDNA from MPS is not present in MB, it is at once included into fraction R (slide *75. nhDNA-MPS*). Further subdivision of general M fraction into non-resident N fraction and mixed RuN fraction takes place algorithmically (Supplement S4, section 5.12).

Let us enumerate the possible reasons for mixed RuN fraction existence:

- blood biomaterial contamination by skin microbiome during venipuncture;
- transport of microorganism and/or its nhDNA from skin into blood during trauma and/or infectious inflammation of derma;
- existence of identical strains in skin microbiome and GIT (URT);
- mapping of different species on a single reference one;

To reduce the size of mixed RuN fraction it is recommended to take skin biopsies from those body parts where the probability of transfer of GIT (URT) microbiome is minimal (i.e. facial skin around nose and mouth, hand skin, skin near anus, etc. are excluded). At the same time it is also important to consider information on Streptococcus sp. biogeography (section 2.3).

As a result of complex study of metagenomes, answers to questions posed in section 3.3 will be received. The final importance of project results depends on the received answers.

3. Objectives, tasks and main questions

3.1. Objectives

Objectives	Tasks	Slides
Studying subprocesses underlying systemic psoriatic process. Determining the role of macromolecular small intestine permeability in PD pathogenesis. Determining the role of whole blood metagenome in PD pathogenesis. Determining the role of PAMP-nemia in PD pathogenesis. Testing basic hypotheses of systemic model of psoriatic disease pathogenesis (H1-1, H2 and HN3).	T1,T2, T3	*22. SPPN-PAMP-nemia* *23. SPPN* *27. NCS1_Hypo*
Complex study of whole blood metagenome and metagenome of psoriatic skin (phagocytes). Studying nhDNA income from systemic blood flow into psoriatic skin. Obtaining facts to support HN10 (HN10-S) hypothesis.	T1,T2, T4,T5	*27. NCS1_Hypo* *28. Biotransfer* *29. HN10-S* *75. nhDNA-MPS*
Summing up and preparing for NCS2 – the second (diagnostic-medical) stage of the project.	All tasks	

To achieve these objectives it is necessary to solve tasks (section 3.2) and receive answers to questions (section 3.3).

Implementing NCS1 project is divided into several stages (slide *84. Part_Order_NCS1*). At the initial stage, besides collecting information on PP-candidates to participate in the project and carrying out selection, it is important to provide development, formation and approbation of IEMC (independently or within acquired Software) specialized for PP. All information (the questionnaire, examination and test results) will be stored in IEMC. Participants will have access only to their IEMC, experts will have access to all IEMC.

3.2. Tasks

Table 8. Tasks.

T1 tasks (practical – pilot stage).	Notes
T1.1. Optimizing patient preparation before blood sampling (diet in the previous days and on the morning of sampling day). This is necessary to achieve the maximum total bacDNA concentration of resident small intestine microbiome in whole blood and psoriatic skin.	This will make it possible to identify minor species of whole blood metagenome in a more precise and cheaper way. It will enable us to include a larger number of samples into the library for one start of productive sequencer and/or to carry out sequencing on a less productive sequencer.
T1.2. Optimizing sampling time of postprandial blood (after the intake of standard breakfast). This is necessary to achieve the maximum total bacDNA concentration of resident small intestine microbiome in whole blood.	
T1.3. The choice of kit and optimizing sample preparation and protocol on DNA isolation from whole blood. This is necessary to ensure the maximum total bacDNA representation in DNA-samples. Criteria: the minimum level of contamination of reagents (task T1.6), DNA fragmentation parameters (median and deviation of distribution).	(section 5.10, p.6)

T1.4. Optimizing the protocol of hDNA elimination from DNA-samples of whole blood for the maximum increase of total bacDNA representation. This also includes optimization of DNA-sample processing: T1.4.1. Studying fragmentary distribution of bacDNA (median and deviation) in DNA-samples. Determining possibility of effective fractional enrichment of bacDNA by separating (all or part of) low-molecular fraction from DNA-samples of blood (instead of NME use). T1.4.2. If fractional enrichment if found inefficient (T1.4.1), exercising removal of fractions with a size of <=15 KB (preparation of DNA-samples for NME use). Optimizing NME protocol.	For blood and NTC. (section 5.8).
T1.5. Developing bacDNA-test – a new way of determining macromolecular small intestine permeability. It is determined by the relation between bacDNA concentration in whole blood taken postprandially (in a fixed time after the termination of standard breakfast) and on an empty stomach.	(section 5.10, p.8) This will make it possible to determine macromolecular small intestine permeability in a new way along with identifying whole blood metagenome.
T1.6. Determining correlation between the maximum total bacDNA concentration in DNA-samples of NTC and the average total bacDNA concentration in DNA-samples of blood. This is essential for taking measures (if necessary) of changing the protocol and/or of reducing contamination level during the main stage of the project.	(section 5.7)
T1.7. Determining degradation rates of bacDNA in whole blood phagocytes. Way of determining: measuring bacDNA concentration in one sample of whole blood previously divided into several equal parts. Measurement is carried out for each part independently after keeping it at 37 C° for a certain time. This will enable us to prepare optimum protocol of DNA isolation from biopsies of psoriatic skin (directly from them or from previously isolated skin phagocytes). The protocol is to provide the maximum total bacDNA representation of non-resident origin in DNA-samples of skin.	If fractional enrichment is successful (task T1.4.1), solving this task will not be necessary, as DNA isolation from skin biopsies and subsequent fractional enrichment of bacDNA will be the optimum protocol (for more detail see section 5.11).

T2 tasks (practical). **Identifying MB – whole blood metagenome.** **Determining PAMP-nemia level.** **Determining macromolecular small intestine permeability.**	**Notes**
T2.1. Developing and using whole metagenomic sequencing (WMS) to detect full nhDNA composition in whole blood to within species, i.e. identifying MB – whole blood metagenome. This includes T2.1.1. Using standard kit to isolate all DNA (chosen according to the results of solving task T1.3). T2.1.2. DNA-sample preparation: hDNA elimination for the maximum increase of total bacDNA representation (the protocol is determined according to the results of solving task T1.4). T2.1.3. Determining concentration of all DNA and bacDNA. T2.1.4. Library formation for DNA-samples of blood and DNA-samples of NTC. T2.1.5. Whole metagenomic sequencing of the library with required total output.	For blood and NTC. Similar to task T4.2.

T2.2. Determining each nhDNA concentration found in DNA-samples by WMS-test results.	For blood and NTC. (section 2.2 and Supplement S2, section 5.12)
T2.3. Determining Qsimp – macromolecular small intestine permeability (OVA test or bacDNA-test).	(section 5.10, p.8)
T2.4. Determining Qlpsb – LPS concentration in biomaterial by one of the standard tests.	(section 5.6)
T2.5. Determining Qsum – PG concentration by one of the standard tests.	(section 5.6)

T3 tasks (analytical). **Studying MB – whole blood metagenome, PAMP-nemia and macromolecular small intestine permeability separately and in complex. Test of hypotheses H1-1, H2 and HN3.**	**Notes**
T3.1. Determining correlations between the maximum total bacDNA concentration in DNA-samples of NTC and average total bacDNA concentration in DNA-samples of blood at the main steps of protocol implementation.	At steps ST1-ST2, ST1-ST3 and ST1-ST4 these correlations should not exceed 0.05-0.1% (control during protocol implementation) (section 5.7).
T3.2. Studying NTC metagenome. Identification of major contaminants according to the results of WMS-tests for DNA-samples of NTC.	
T3.3. Correction of MB – whole blood metagenome according to the results of solving tasks T3.1 and T3.2. This is carried out on major contaminants and species whose concentration in MB proved to be the same or lower than the maximum total bacDNA concentration in DNA-samples of NTC.	
T3.4. Determining Qminus – total bacDNA concentration of Gram(-) species responsible for LPS-load.	
T3.5. Determining Qlpsm – total bacDNA concentration of species responsible for TLR4-active LPS-load. With sufficient coverage – by identifying genes responsible for TLR4-activity of LPS. Qlpsm calculation (possible with the use of TLR4-activity coefficients). Coordination of Qminus, Qlpsm and Qlpsb (see task T2.4).	With insufficient coverage – determining Qlpsm through representation of genera nearly all species of which have LpxM and LpxL genes.
T3.6. Determining Qplus – total bacDNA concentration of Gram+ species (responsible for PG-load). Coordination of Qplus and Qpgb (see task T2.5).	
T3.7. Determining Qpsb – total bacDNA concentration of all species having interpeptide bridges IB-Y. With sufficient coverage – by determining the presence of MurM, MurN genes responsible for the formation of interpeptide bridges IB-Y in peptidoglycan. Species having these genes (both of them simultaneously), are presumed psoriagenic.	With insufficient coverage – Qpsb is to be used: information on representation of species determined in advance (section 5.4). If this occurs, the opportunity to identify new psoriagenic species as well as splitting species into strains depending on the presence of MurM, MurN genes is lost.
T3.8. Search of direct correlation between Qsimp - macromolecular small intestine permeability and PD severity on PASI. Comparison of Qsimp in PP and HP. Test of hypothesis H1-1.	Discovering correlation of Qsimp with PD severity and its excess in PP (compared to HP) will confirm H1-1 – one of the basic hypotheses of systemic model of pathogenesis.

T3.9. Search of direct correlations between bacDNA concentration in whole blood: Qminus (Gram(-) species), Qlpsm (Gram(-) TLR4-active species), Qplus (Gram(+) species) and Qpsb (species presumed psoriagenic) and PD severity on PASI. Comparison of these parameters for PP and HP. Metagenomic test of hypotheses H2 and HN3.	Discovering correlations with PD severity and their excess in PP (compared to HP) will confirm H2 and HN3 – the basic hypotheses of systemic model of pathogenesis.
T3.10. Search of direct correlations between Qlpsb and Qpgb parameters and PD severity on PASI. Comparison of these parameters for PP and HP. Biochemical test of hypothesis HN3.	Discovering correlations with PD severity and their excess in PP (compared to HP) will confirm HN3 – a hypothesis of systemic model of pathogenesis.
T3.11. Search of complex correlations of the set of the above listed parameters (and, probably, of some others) with PD severity on PASI when solving tasks T3.8, T3.9 and T3.10. Firstly, according to YN-model of pathogenesis, SPPN severity is determined by several parameters (Example 1). Secondly, what if YN-model will require adjustment according to the received results? For example, if correlation between PD severity and concentration of bacDNA Staph.aures in whole blood is discovered (Example 2).	Example 1. The same PD severity is possible at (high Qpsb + low Qlpsm and Qlpsb) and at (low Qpsb + high Qlpsm and Qlpsb). Example 2. Staph.aures aggravates PD severity if it is present on plaques. But its peptidoglycan is not similar to peptidoglycan of Str.pyogenes and does not contain presumed Y-antigen…
T3.12. Determining plastome in whole blood metagenome. Plastome species are the basis for non-resident fraction of MPS (tasks T5.4 and T5.5).	(section 5.9)

T4 tasks (practical). **Identifying MPS – metagenome of psoriatic skin (phagocytes).**	**Notes**
T4.1. Developing and implementing immunomagnetic method of phagocyte isolation from psoriatic skin (section 5.11).	See task T1.7: its solution will show whether it is necessary to solve this task or not.
T4.2. Developing and implementing whole metagenomic sequencing (WMS) to discover nhDNA composition of psoriatic skin (phagocytes) to within species, i.e. identifying MPS. This includes T4.2.1. Implementing the protocol of forming DNA-samples of skin where total bacDNA representation of non-resident origin will be the highest possible. T4.2.2. Preparation of DNA-samples: hDNA elimination for the maximum increase of total bacDNA representation. T4.2.3. Determining concentration of all DNA and bacDNA. T4.2.4. Library formation for DNA-samples of skin and DNA-samples of NTC. T4.2.5. Whole metagenomic sequencing (WMS-test) of library with required total output.	For skin and NTC. Similar to task T2.1.
T4.3. Determining concentration of each nhDNA found in metagenome (solved in the same way as task T2.2).	For skin and NTC.

T5 tasks (analytical). **Complex study of MB – whole blood metagenome and MPS – metagenome of psoriatic skin (phagocytes).** **Test of hypothesis HN10-S.**	**Notes**
T5.1. Determining correlations between the maximum total bacDNA concentration in DNA-samples of NTC and the average total bacDNA concentration in DNA-samples of skin at the main steps of protocol implementation.	At steps ST1.4, ST2.2 and ST2.4 these correlations should not exceed 0.05-0.1% (control during protocol implementation) (sections 3.2.1, 5.7).
T5.2. Studying NTC metagenome. Identification of major contaminants according to the results of WMS-tests for DNA-samples of NTC.	(section 5.7)
T5.3. Correction of MPS – metagenome of psoriatic skin (phagocytes) according to the results of solving tasks T5.1 and T5.2. This is carried out on major contaminants and species whose concentration in MPS proved to be the same or lower than the maximum total bacDNA concentration in DNA-samples of NTC.	
T5.4. Determining plastome in MPS – metagenome of psoriatic skin (phagocytes). Plastome species are a good basis for identifying N – non-resident fraction of MPS (task T5.5).	Integrated with task T3.12. (section 5.9)
T5.5. Complex study of MB – whole blood metagenome and MPS – metagenome of psoriatic skin (phagocytes). Determining MPS fractions: R – resident and M – general. Division of M – general fraction into N – non-resident and RuN – mixed. Determining representation and concentration of nhDNA in each fraction. Determining bacDNA representation and concentration of pathogenic and presumed psoriagenic bacteria of resident and non-resident origin in each fraction. Test of hypothesis HN10-S. (slides *28. Biotransfer*, *29. HN10-S* and *75. nhDNA-MPS*).	Discovering and determining characteristics of N – non-resident fraction (as well as of the whole non-resident subset in MPS) will confirm hypothesis HN10-S (and, indirectly, HN10). (Supplements S4 and S5, section 5.12)
T5.6. Search and study of correlations between PD severity and parameters, characterizing composition and concentration of bacDNA in MB and MPS.	
T5.7. A similar approach if considerable concentration of non-bacterial nhDNA (archean, fungi, plants, helminths, viruses, etc.) in MPS is detected.	Virome of psoriatic skin has not been studied before (Yan 2017).
T6 tasks (final). T6.1. Statistical analysis and assessment of results. T6.2. Report preparation. T6.3. Preparation and publication of articles. T6.4. Completion and preparation of the second (diagnostic-medical) stage of the project.	

3.2.1. Task solution order

Further are formulated consecutive steps (ST) which are to be taken in order to solve the above listed tasks.

ST1. Formation of DNA-samples (and determining their characteristics) by DNA isolation from
ST1.1. Whole blood (tasks T1.3 and T2.1.1)
ST1.2. Psoriatic skin (from phagocytes or from biopsy as a whole – the decision taken after the pilot research) (tasks T1.4.1, T1.7 and T4.2.1)

ST1.3. NTC (to assess contamination through the whole protocol it is necessary to pass several NTC) (tasks T2.1 and T4.2)
ST1.4. Determining concentration of all DNA and bacDNA. bacDNA concentration – e.g. by Femto Bacterial DNA Quantification Kit. (tasks T2.1.3 and T4.2.3)

ST2. Enrichment of nhDNA in DNA-samples (representation increase)
(tasks T2.1.2, T4.2.2, T2.1.3, T4.2.3)
ST2.1. Preparation for using NME (NebNext microbiome enrichment): removal of DNA fractions with a size of <= 15 KB (division of fractions by means of electrophoresis with agarose gel).
ST2.2. Determining concentration of all DNA and bacDNA.
ST2.3. Maximum elimination of hDNA by NME.
ST2.4. Determining concentration of all DNA and bacDNA.

In case of successful development of fractional nhDNA enrichment at the pilot stage (task T1.4.1), there will be only one step instead of steps ST2.1, ST2.2 and ST2.3:
ST2.1N. Fractional nhDNA enrichment in DNA-samples (electrophoresis with agarose gel).

ST3. Ensuring low contamination level at ST1.4, ST2.2 and ST2.4 (the relation of the maximum bacDNA concentration in DNA-samples of NTC to the average level of bacDNA concentration in DNA-samples of blood and skin), not higher than 0.05-0.1% (tasks T3.1 and T5.1).

ST4. Library formation for whole metagenomic sequencing
ST4-1. For DNA-samples (of blood and NTC) (T2.1.4 task).
ST4-2. For DNA-samples (of skin and NTC) (T4.2.4 task).

ST5. Whole metagenomic sequencing with required total output.
Two consecutive starts of the sequencer:
ST5.1. For ST4-1 library (task T2.1.5)
ST5.2. For ST4-2 library (task T4.2.5).
The necessary total outputs are determined by the pilot stage results.

During the pilot stage only ST1-ST3 steps for PP blood samples and samples of NTC are carried out, i.e. without forming libraries and sequencing. Note that all tasks T1 are to be solved.

3.3. Basic questions

Table 9. Questions and answers.

Questions	Possible answers	Information
Q1.1. Does PD severity correlate with any nhDNA concentration in whole blood? (slide *85. Stage1-Q1*)	**Yes** - Direct confirmation of H2 hypothesis and possibly of HN3 hypothesis. **No** – See Note 1.1.	Tasks T3.6 and T3.8.
Q1.2. Does PD severity correlate with PAMP-nemia level? (slide *85. Stage1-Q1*)	**Yes** - Direct confirmation of HN3 hypothesis. **No** – See Note 1.2.	Tasks T3.7 and T3.8.
Q1.3. Does PD severity correlate with increased macromolecular small intestine permeability?	**Yes** - Direct confirmation of H1-1 hypothesis. **No** – See Note 1.3.	Task T3.5.
Q2.1. Does non-degraded nhDNA from blood come into psoriatic skin? (slide *86. Stage1-Q2*) The same question formulated in other words: Is N – non-resident fraction of MPS – metagenome of psoriatic skin (phagocytes) found? (slides *75. nhDNA-MPS* and *76. MPS-example*)	**Yes** - This fact proves HN10-S hypothesis and supports HN10 hypothesis. **No** – See Note 2.1.	Task T5.2.
Questions Q2.2-Q2.3 only make sense in case of the answer "Yes" to question Q2.1.		

Questions	Possible answers	Information
Q2.2. . What does non-resident fraction N represent, what is its composition and representation in MPS? Is the composition of fraction N similar to the composition of fraction MB? Coefficient of variation for fraction N < 1/3 (i.e. is the distribution homogeneous?)	**Yes** - This fact supports HN10 hypothesis. **No** – See Note 2.2.	(Supplements S4 and S5, section 5.12)
Q2.3. Is there bacDNA of species presumed psoriagenic and/or pathogenic bacteria in non-resident fraction N? In what concentration?	**Yes** - This fact supports H2 and HN3 hypotheses; **No** - See Note 2.3.	

Notes (Table 9)

1.1.1. There is a possibility of a negative answer to Question Q1.1 as there are very few studies, in which bacDNA concentration in whole blood is determined (section 2.2). All of them were conducted with the use of 16S-test (which does not make it possible to identify bacterial species, whereas information on bacterial genera has appreciable error). Only one of them (Okubo 2002, slide *31. Okubo*) dealt with psoriatic patients and used blood monocytes as biomaterial (but metagenome was not studied).

1.1.2. A negative answer to Question Q1.1 is possible, if for the most PP the maximum of bacDNA concentrations (pathogenic and/or presumed psoriagenic species) in whole blood is the same or lower than contamination level. In this case it is necessary to take measures to reduce contamination level and to perform the whole protocol anew (section 5.10).

1.1.3. Should we look for correlations of PD severity for the same parameters of average MB – whole blood metagenome determined by mixture of several blood samples, taken from the patient within a certain period? For more detail see Supplement S3 (section 5.12).

1.1.4. A negative answer to Question Q1.1 is possible in case of a highly improbable event. more specifically, if PP group in the project happens to include PP with very high GRS (genetic risk score) of psoriasis development. PASI of such PP to a greater extent depends on genetic predisposition than on concentration of bacterial products from pathogenic and/or presumed psoriagenic bacteria in whole blood (i.e. there is a strong reaction even to their small concentrations). There are several techniques of GRS calculation depending on the chosen gene pattern and assessment of their role in PD pathogenesis (Kisiel 2017, Tsoi 2017). Research results and, as a consequence, technique of GRS calculation depend on the residence area (GRS calculations for PP have never been made in Russian Federation). It is known that there is correlation between several SNP (Single nucleotide polymorphism) and PD severity, whereas for GRS such correlation has not been established (Nikamo 2015, Yin 2015).

This situation may involve the following solution:

- Choosing a method of GRS calculation (e.g. one used by 23andMe).
- Adapting it to the information on gene role for PP in Russian Federation (Kubanov 2014).
- Determining GRS for all PP.
- Attempting to discover correlation between PASI and combined parameters: GRS and total bacDNA concentrations of pathogenic and/or presumed psoriagenic species in whole blood (virtually solving task T3.8 again with one more GRS parameter included).

1.1.4. If contamination level has not exceeded admissible (par. 1.1.2) and the attempt to consider GRS parameter (par. 1.1.3) has not been successful, it is necessary to try modifying YN-model of pathogenesis (with regard to hypotheses H2 and HN3) on the basis of the obtained results. This modification is either to provide affirmative answers to Questions Q1.1 and Q1.2 or to offer convincing explanation why these answers (one or both) were negative.

1.2. A negative answer to Question Q1.2 is virtually impossible as several studies with a rather large PP and HP group received results presupposing only an affirmative answer (Garaeva 2007, Gumayunova 2009a, Gumayunova 2009b, Gumayunova 2009c). See slides *9. SIBO-2*, *10. SIBO_Moscow* also.

1.3. A negative answer to Question Q1.3 is highly improbable as several studies with a rather large PP and HP group received results presupposing an affirmative answer (Rudkovskaya 2003, Stenina 2004, Harkov 2005, Harkov 2006, Harkov 2008), see also slide *7. Permeability-2*.

2.1.1. A negative answer to Question Q2.1 is possible, if for most PP the maximum of bacDNA concentrations coming from blood to psoriatic skin (in blood phagocytes) is the same or lower than contamination level. If contamination level has exceeded admissible, it is necessary to take measures to reduce contamination level and to perform the whole protocol anew.

2.1.2. If contamination level has not exceeded admissible, an attempt has to be made to modify YN-model of pathogenesis (with regard to hypothesis HN10) on the basis of the obtained results. This modification is to provide an affirmative answer to Question Q2.1 or to offer convincing explanation why this answer was negative.

The procedure for questions Q1.1, Q1.2 and Q2.1 is illustrated in slide *87. Stage1-Q1&2*.

2.2. Should we compare composition of fraction N to average MB – whole blood metagenome, determined by mixture of several blood samples taken from the patient within a certain period preceding skin biopsy sampling? For more detail see Supplement S3 (section 5.12).

2.3. Is the sampling too limited (2-3 biopsies from PP)? Presence of bacDNA of a particular species in a small sampling may not correlate with LPS, PG and PG-Y presence. In this case it is necessary to take more biopsies from several characteristic sites from one PP (5-10 biopsies). All biopsies from one PP are studied as single biomaterial.

4. Conclusion

Scientific novelty of NCS1 project consists in testing the main hypotheses of systemic YN-model of PD pathogenesis. Notably, the newest methods of research will be used for this purpose: whole metagenomic sequencing of whole blood and psoriatic skin (phagocytes), as well as determining macromolecular small intestine permeability by bacDNA-test (slide *88. Stage1-new* and Table 10). Project novelty is also evaluated by comparison (Table 4, Table 7).

Table 10. Researches to be carried out for the first time.

Title	Patients	Note
Parameters of fragment distribution of bacDNA, found in DNA-samples from whole blood are determined.	PP and HP	For the first time.
Whole blood metagenome is identified by whole metagenomic sequencing method.	PP and HP	For the first time.
Whole blood plastome (as part of its metagenome) is identified.	PP and HP	For the first time.
Metagenome of psoriatic skin (phagocytes) is identified by whole metagenomic sequencing method (including its non-resident fraction).	PP	For the first time.
nhDNA concentration in whole blood is determined.	PP and HP	For the first time for PP.
nhDNA concentration of psoriatic skin (phagocytes) is determined (including of non-resident fraction).	PP	For the first time for PP.
Complex study of whole blood metagenome and metagenome of psoriatic skin (phagocytes) is carried out.	PP	For the first time.
Macromolecular small intestine permeability is determined by bacDNA-test.	PP and HP	For the first time.
Main PAMP concentration in blood is determined.	PP and HP	PG – for the first time.

Successful implementation of NCS1 (the first diagnostic stage of the project) will create prerequisites for implementing NCS2 (the second diagnostic-medical stage of the project): "Development and use of PD treatment technology based on stable correction of small intestine and pharynx microbiomes and macromolecular small intestine permeability aiming to achieve long-term and stable remission", as it represents the objective of the project as a whole.

5. Appendices

5.1. Abbreviations and terms

		Description
AMP		Antimicrobial proteins (peptides)
bacDNA		Bacterial DNA
BC		Buffy coat. The fraction of leukocytes and platelets which is formed between erythrocytes and plasma after whole blood fractionation.
DB		Data Base
DC		Dendritic cells
DC-Y		(PG-Y)+DC - Dendritic cells repleted by PG-Y
DC-T	●	Tolerized DC.
DC-R	●	Dendritic cells, which reprogrammed (tolerized) and repleted by PG-Y are subfraction of tolerized fraction DC-T.
GIT		Gastro-Intestinal Tract
IB-Y		Interpeptide bridges of peptidoglycan Str.pyogenes: (L-Ala)-(L-Ala) or (L-Ser)-(L-Ala).
IEMC		Integrated Electronic Medical Card
hDNA		Host DNA (in this project human DNA)
HP		Healthy person
hRNA		Host RNA (in this project human RNA)
HPV		Human Papilloma Virus
KC		Keratinocytes
kPAMP		Key PAMP: LPS, PG and (in YN-model) bacDNA.
LPS		Lipopolysaccharide
maDC-Y		Mature DC derived from DC-R or from MoDC-R, presenting Y-antigen
MB		Whole blood metagenome - set of all nhDNA, contained in whole blood.
MDP		Muramyl dipeptide - component Gram+ and Gram(-) PG, ligand of NOD2
Metagenome		Set of all nhDNA (non-host DNA, i.e. in this project of non-human one), contained in biomaterial.
MF		Macrophages derived from Mo or from MoDP
MF-T	●	Macrophages derived from Mo-T
MF-R	●	Macrophages derived from Mo-R
MHC		Major histocompatibility complex
Mo		Monocytes
Mo-Y		(PG-Y)+Mo - Monocytes repleted by PG-Y
Mo-T	●	Tolerized Mo
Mo-R	●	Monocytes, which reprogrammed (tolerized) and repleted by PG-Y, are a subfraction of tolerized fraction Mo-T.
MoDP		Skin resident stem cells - precursors of part of MoDC and MF
MoDC		DC derived from Mo or from MoDP (slide *29. HN10-S*)
MoDC-Y		(PG-Y)+MoDC - MoDC repleted by PG-Y
MoDC-T	●	DC derived from Mo-T
MoDC-R	●	DC-R derived from Mo-R
MPS		Metagenome of psoriatic skin (phagocytes*) - set of all nhDNA from psoriatic skin (phagocytes*). * The decision whether phagocytes from skin biopsies will be isolated (for subsequent DNA isolation from them) or DNA will be directly isolated from biopsies, is taken after the pilot stage.
Neu		Neutrophils
Neu-Y		(PG-Y)+Neu - Neutrophils repleted by PG-Y
NET		Neutrophil extracellular traps (= netosis products) - formed at netosis (slide *61. Netosis*).

		Description
NCS		Nature Clean Skin – name of the project consisting of two stages: NCS1 (diagnostic) – described here, and NCS2 (diagnostic-medical) – will be subsequently described in more detail.
nhDNA		Any non-host DNA (here, non-human) – from bacteria, archean, fungi, plants, helminths, viruses, phages, etc.
NLS		Non-lesional (prepsoriatic, uninvolved) skin – PP skin without symptoms of psoriasis
NME		NebNext Microbiome Enrichment. Kit for hDNA elimination and, as a result, enrichment of nhDNA representation.
NOD1		Intracellular receptor - ligand to DAP (slide *15. PAMP,TLR,NOD*, Pashenkov 2018)
NOD2		Intracellular receptor - ligand to MDP (slide *15. PAMP,TLR,NOD*, Pashenkov 2018)
NTC		No Template Control. Control samples which do not contain DNA expected to be found in the main samples. Applied to determine contamination level and particular contaminants (section 5.7).
PAMP		Pathogen-associated molecular patterns. (in particular LPS, PG, bacDNA and (1,3)-beta-D-glucan) (Fukui 2016) (slide *15. PAMP,TLR,NOD*).
PAMP-nemia		Definition in YN-model: Chronic increase of kPAMP-load (binding, endocytosis) on blood phagocytes resulting in - increasing of kPAMP concentration in blood; - increased kPAMP-carriage of blood phagocytes. The main kPAMP are LPS, PG and bacDNA. (slides *22. SPPN-PAMP-nemia*, *23. SPPN*, section 5.3.1.). Definition in Y-model (Table 11, SP4)
PASI		Psoriasis Area and Severity Index
PD		Psoriatic disease
PDC		Plasmacytoid dendritic cells
PG		Peptidoglycan. Any peptidoglycan (including PG-Y)
PG-Y		Peptidoglycan A3alpha with interpeptide bridges IB-Y (but can also contain others bridges)
PLS		Psoriatic lesional skin
PP		Psoriatic patient
PsB		Psoriagenic bacteria - species of bacteria presumed psoriagenic (with PG-Y peptidoglycan)
Representation		Relative presence (percent share) of something in biomaterial (sample).
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.
SPP		Systemic psoriatic process (basis of Y-model of pathogenesis) (Peslyak 2012a)
SPPN		Systemic psoriatic process in YN-model - differs from SPP (section 5.2) (slides *22. SPPN-PAMP-nemia*, *23. SPPN*).
SVLD		Sterile variant of low-microbial diet
TL		T-lymphocytes
TL-Y		Y-specific TL (have receptors ligandic to Y-antigen epitopes)
TLR2		Membranous receptor - ligand to PG-fragments LTA, BLP
TLR4		Membranous receptor - ligand to LPS
TLR7		Endosomal receptor – ligand to ssRNA, bacRNA, but also and to hRNA-LL37 complexes.
TLR8		Endosomal receptor – ligand to ssRNA, bacRNA, but also and to hRNA-LL37 complexes.
TLR9		Receptor - ligand to CpG - fragment of bacterial or virus DNA. As a rule, intracellular (endosomal), but at neutrophils also expressed on membrane (Lindau 2013). (slide *15. PAMP,TLR,NOD*)
URT		Upper Respiratory Tract
WMS		Whole metagenome sequencing (shotgun)
Y-antigen		part(s) of interpeptide bridge IB-Y (sections 5.4, 5.5)
Y-model		model of psoriasis pathogenesis proposed in the monograph (Peslyak 2012a, Peslyak 2012b)
YN-model		model of psoriasis pathogenesis proposed in this study

The words in bold denote common abbreviations.

● – used only in Y-model.

5.2. YN-model of pathogenesis. Comparison with Y-model.

The given section outlines the main differences between YN-model and Y-model. Y-model of pathogenesis presupposes the key role of blood phagocytes (monocytes and dendritic cells) in the development and support of psoriatic plaques (Peslyak 2012a, Peslyak 2012b). Within Y-model it is tolerized monocytes and dendritic cells that bring non-degraded bacterial products, LPS and PG (including PG-Y) into psoriatic derma. In Y-model the role of blood neutrophils was recognized only as auxiliary, as one of intermediaries responsible for bone marrow preparation of monocyte and dendritic cell progenitors at hemopoiesis stage (Peslyak 2012a, App.5). The role of neutrophils attracted to psoriatic skin was regarded as that of active (but not basic) participants of innate immune response to initiating process LP2, which corresponds to their role in other models of pathogenesis, particularly in GL-model (Gilliet 2008).

Such an approach was based on interpreting information from (Numerof 2006), which discussed an ineffective experiment on intravenous administration of antibodies to CXCL8 (IL-8) (described in detail in Krueger 2002). At the highest dose (3 mg/kg of weight) it resulted in 50% PASI decrease in 30% of PP. A good review in (Sabat 2007) also describes the same experiment as completely unsuccessful, since no changes in PASI were allegedly registered. This review also mentioned decrease of neutrophil concentration in psoriatic skin during this experiment (Krueger 2002 and Numerof 2006 give no information about that). There were no other significant studies of the role of neutrophils in psoriasis pathogenesis, known at the time of monograph preparation (Peslyak 2012a, Peslyak 2012b).

Other models of pathogenesis (Gilliet 2008, Guttman-Yassky 2011, Perera 2012, Tonel 2009) assign an important role to neutrophils in psoriasis pathogenesis. This primarily involves active LL37 secretion, which is regarded as a link of the vicious cycle. In Y-model, LL37 secretion by neutrophils is part of LP3 process, it definitely affects initiation and support of LP4, but is not regarded as a link of the vicious cycle (Table 11).

The results of recently published works devoted to studying neutrophils at psoriasis, make us reconsidering their role in pathogenesis, and formulate new YN-model of pathogenesis. Its major differences are connected with the fact that, after being attracted into psoriatic skin, an essential part of neutrophils undergo netosis and, as this occurs, netosis products appear in extracellular space (Lin 2011, Skrzeczynska 2012, Lowes 2014, Hu 2016, Schon 2017, Schon 2018, slide *61. Netosis*). For more detail see section 5.3.

All differences between the two models are given in Table 11. In Y-model, the leading role of Y-antigen delivery to psoriatic skin is assigned to tolerized monocytes and dendritic cells, whereas in YN-model this role is attributed to neutrophils, part of which undergo netosis after being attracted into skin. YN-model follows the basic structure of Y-model and uses its designations and numbering of processes and subprocesses. New subprocess LP3a (netosis of some Neu and Neu-Y) is included in vicious cycle B (slides *25. Local_processes_YN* and *26. YN-model*).

Process LP5 and subprocesses LP6.4, LP7.1 in Y-model were necessary for psoriatic plaque initiation since subprocess LP6.1 (loss of tolerance to kPAMP at Mo-T and DC-T) could start only after LP5 (adaptive response against LP2). In YN-model, process LP5 and subprocesses LP6.4, LP7.1 are possible, but not obligatory, and are therefore not included in YN-model scheme. In YN-model, an obligatory condition of psoriatic plaque initiation is transit process LP4 (trigger of adaptive response), whereas subprocess LP3a (netosis of some Neu and Neu-Y) consists in LPN3 (innate response) and is the key link of vicious cycle B.

YN-model is more elegant and more appropriate than Y-model. These models do not contradict each other. These models do not contradict each other. Hypotheses tested within this project will not enable us to tilt in favour of one of the models. Indeed, H1-1 and H2 are shared by both models, while HN3 and HN10 are H3 and H10 expansions respectively (Supplement S6, section 5.12).

If the project is successfully implemented, a more exact definition of pathogenesis model will be given in the course of future research.

Table 11. Y-model and YN-model. Processes and subprocesses.

Y-model	YN-model.
Systemic	
SPP. Systemic psoriatic process. Increased kPAMP-carriage of tolerized phagocytes. Increased (PG-Y)-carriage of R-phagocytes.	**SPPN.** Systemic psoriatic process. Increased kPAMP-carriage and particularly (PG-Y)-carriage of blood phagocytes (slides *22. SPPN-PAMP-nemia*, *23. SPPN)*
SP1. Increased small intestine permeability for fragments of bacterial products with PAMP (including kPAMP).	yes
SP2. Growth of populations of Gram(-) TLR4-active and Gram+ NOD2-active bacteria on small intestine mucosa.	yes
SP2.1. Growth of populations of psoriagenic PsB on small intestine mucosa.	yes
SP3. Disturbance of production and/or circulation of bile acids.	yes
SP4. PAMP-nemia: Chronic increase of kPAMP-load (binding, endocytosis) on blood phagocytes resulting in a) formation fraction of tolerized phagocytes; b) increase of kPAMP concentration in blood; c) increased kPAMP-carriage of tolerized phagocytes. The main kPAMP are PG and LPS.	**SPN4.** PAMP-nemia: Chronic increase of kPAMP-load (binding, endocytosis) on blood phagocytes resulting in - increase of kPAMP concentration in blood; - increased kPAMP-carriage of phagocytes. The main kPAMP are LPS, PG and bacDNA. (section 5.3.1.). Formation of the fraction of tolerized monocytes Mo-T and dendritic cells DC-T (which are kPAMP-carriers) in blood is possible, but not obligatory.
SP4.1. (PG-Y)-nemia.	yes
SP5. Overload and/or disorders of detoxication systems	yes
SP6. Tonsillar PsB-infection	yes
SP7. Deviation in intracellular signal path "from MDP recognition through NOD2-ligand to chemostatus change" (<1%).	no
SP8. Growth of tolerized fractions Mo-T and DC-T. Increased kPAMP-carriage of these blood phagocytes.	**SPN8.** Increased kPAMP-carriage of blood phagocytes. (*section 5.3.1)*
SP8.1. Growth of subfractions Mo-R and DC-R. Increased (PG-Y)-carriage of these blood phagocytes.	**SPN8.1.** Increased (PG-Y)-carriage of blood phagocytes. (*section 5.3.1)*
Local	
LP1. Attraction of immunocytes from blood flow.	yes
LP1.1. Attraction of Mo and DC, Mo-T and DC-T (incl. Mo-R and DC-R) from blood flow.	**LP1a.** Attraction of non-lymphocytic immunocytes (Neu, Neu-Y, Mo, DC, PDC, NK, etc.) from blood flow.
LP1.2. Attraction of other immunocytes from blood flow.	**LP1b.** Attraction of T-lymphocytes from blood flow.
LP2. Initiating and aggravating process. LP2 (IN) and LP2(HPV) are detailed in (Peslyak 2012b)	yes
LP2(IN). Open trauma of derma	yes
LP2(HPV). HPV-carriage of keratinocytes	yes
LP3. Innate response against LP2	**LPN3.** Innate response, including:
	LP3a. Netosis of some Neu and Neu-Y. Netotic products NET, including Y-antigens, get into extracellular space. (slides *25. Local_processes_YN* and *26. YN-model*).
LP3.1. Formation of hDNA-LL37 and hRNA-LL37 complexes	**LP3b.** Formation of hDNA-LL37 and hRNA-LL37 complexes

Y-model	YN-model.
LP4. Trigger of adaptive response against LP2	**LP4.** Trigger of adaptive response
LP5. Adaptive response against LP2	Possible, but not obligatory.
LP6. Mo and DC transformations	**LPN6.** Mo and DC transformations
	LP6a. MF, Mo and DC endocyte netotic products. Phagocytes which endocyted Y-antigens are labeled MF-Y, Mo-Y and DC-Y (slides *25. Local_processes_YN* and *26. YN-model*).
LP6.1. Loss of tolerance to kPAMP: at Mo-T, DC-T, MoDC-T (including Mo-R, DC-R, MoDC-R)	Possible, but not obligatory.
LP6.2. MF and MoDC formation: from any Mo and from Mo-T, Mo-R	**LP6b.** MF and MoDC formation: from any Mo and from Mo-Y
LP6.3. maDC-Z formation: from any DC, MoDC and from PG-Y(-)DC-T, PG-Y(-)MoDC-T	Possible, but not obligatory.
LP6.4. maDC-Y formation: from DC-R, MoDC-R	**LP6c.** maDC-Y formation: from DC-Y and MoDC-Y
LP7. Lymph nodes. Clonal proliferation.	yes
LP7.1. TL-Z formation	Possible, but not obligatory
LP7.2. TL-Y formation	yes
LP8. False adaptive response to imaginary PsB-infection.	yes
LP8.1. Y-antigen presentation by maDC-Y to effector TL-Y.	yes
LP8.2. Keratinocyte hyperproliferation. Change of skin architecture. Growth of basal membrane area and vascularity increase.	yes

5.3. Neutrophils in YN-model

The given section specifies arguments in favor of the changed processes (compared to Y-model) and of the new processes and subprocesses of YN-model of pathogenesis. As these changes and additions are connected with neutrophils, the section is called accordingly.

5.3.1. Blood neutrophils

In the text above, the reasons why neutrophils, apart from blood monocytes and dendritic cells, play a major role in YN-model are enumerated. The requirement of blood phagocyte tolerization which was crucial for Y-model (for monocytes and dendritic cells) is also omitted. Activated blood neutrophils (unlike monocytes and dendritic cells) retain the opportunity of being attracted to inflamed tissues, as LPS impact on them, albeit reduces CXCR4 expression, at the same time contributes to considerable CCR2 expression (Shen 2017).

Senescent neutrophils can take endocyted (in blood flow) bacterial products into bone marrow (which is stimulated by growth of CXCR4 expression), so these products find themselves in extracellular space after apoptosis of these neutrophils. Notably these bacterial products retain their PAMP properties. Such a function of senescent neutrophils is meant for training monocyte progenitors during their maturing in bone marrow (Tacke 2006, Rankin 2010).

During systemic psoriatic process SPPN (due to chronic kPAMP-load) some part of senescent blood neutrophils (along with other neutrophils) is constantly attracted into inflamed psoriatic skin instead of bone marrow. It happens due to constant CCR1 expression and probably owing to change of CXCR4 expression to CCR2 expression (Uhl 2016, Ortmann 2018). In psoriatic skin CCL2, CCL5 (CCR1 ligands) and CCL2, HBD2 and HBD3 (CCR2 ligands) are actively secreted (Table 12).

As a result, these senescent blood neutrophils appear in the place, where they were not supposed to appear, at the same time (presumably) retaining non-degraded bacterial products in them. Due to the fact that there are many factors of netosis in psoriatic skin (Table 13), there occurs netosis of part of these neutrophils (section 5.3.3), especially since senescent neutrophils have a greater potential to netosis (Ortmann 2018).

In YN-model the list of the main kPAMP is extended: bacDNA is added to LPS and PG. This is done because neutrophils have not only endosomal, but also membrane TLR9 receptor (Lindau 2013, slide *15. PAMP,TLR,NOD*). That means that bacDNA (as well as LPS) can contribute to activating (transforming to

prenetotic state) neutrophils even at external contact or binding. Activation intensity largely depends on the percentage of CpG fragments in bacDNA, depending on bacterial species (Dalpke 2006). Interaction of CpG fragments with TLR9 also reduces the tendency of neutrophils to apoptosis (Jozsef 2004), which can contribute to increasing the proportion of neutrophils terminating their existence by netosis.

Section 5.3.3 contains detailed analysis of factors (real and presumed) contributing to netosis at psoriasis (Table 13). Among them there is no bacDNA, as at the moment there are no published studies of bacDNA influence on netosis.

By the results of whole blood WMS-test and determining bacDNA concentration, total concentration of CpG fragments will be determined. This will make it possible to estimate total impact on TLR9 receptor in the same way as estimating total impact on TLR4 receptor (section 5.6).

Intensity of phagocyte activation under the influence of several PAMP considerably increases (synergic effect). There is also synergic influence of a few factors (not only PAMP) on netosis (Table 13). Within the project it is expected to estimate synergic effect on systemic psoriatic process SPPN for three kPAMP (LPS, PG and bacDNA).

5.3.2. Attraction of neutrophils into skin from blood flow

Neutrophils constitute the greatest part of blood phagocytes (> 85%) and are responsible for endocytosis of most bacterial products (Mayadas 2014). To obtain maximally representational blood metagenome, it is necessary to extract nhDNA from all phagocytes.

In healthy skin, neutrophils are virtually absent (Kabashima 2016, Gaspari 2017, slide 59. Neutrophils). They are attracted into skin at the earliest stage of psoriatic plaque initiation (even before visible skin change). They are especially numerous in primary pinpoint psoriatic plaques (Van de Kerkhof 2007, Christophers 2014).

Their essential part can be grouped in the top layers of epidermis, forming Munro's abscesses (slide *60. Neutrophils-Munro*). In chronic psoriatic plaque, neutrophils constitute the greatest part of skin phagocytes (up to 45% at moderate-severe psoriasis), and nearly all of them have non-resident origin (slide *56. Phagocytes-1*, Gottlieb 2005, Fuentes-Duculan 2010, Zaba 2009a).

Prepsoriatic skin adjacent to active plaques and early plaques, is characterized by the presence of CD15+Neu (Albanesi 2009, Albanesi 2010).

The supposition that attraction of blood neutrophils to psoriatic skin under the influence of CXCL8 (IL-8) chemokine, and subsequent secretion of the same chemokine by neutrophils, are links of the vicious cycle, was made long ago (Terui 2000, Gilliet 2008). In other models of pathogenesis Guttman-Yassky 2011, Perera 2012, Tonel 2009), analyzed in detail in (Peslyak 2012b), neutrophil attraction to psoriatic skin was not included into the vicious cycles. LL37 secretion by neutrophils was regarded as link of the vicious cycle, though.

After the discovery that part of neutrophils in psoriatic skin undergo netosis (slides *65. Net-skin-1*, *66. Net-skin-2* and *67. KB-schema*, Lin 2011, Skrzeczynska 2012), it was discussed by the authors of GK-model (Lowes 2014, Hawkes 2017, Laboratory for Investigative Dermatology, The Rockefeller University, New York, USA), but netosis was not included in their pathogenesis model (slide *70. GKH-model*). The scheme of GKH-model is simplistic, compared to GK-model scheme. An even stricter approach is chosen by the authors of a detailed review devoted to psoriasis pathogenesis (Benhadou 2018): they do not only leave out netosis, but also neutrophils from their pathogenesis model (slide *71. BMM-model*). The BMM-model is virtually a simplified GK-model.

Attraction of neutrophils into skin and their netosis have recently been included in the vicious cycle by authors of the following pathogenesis models (slide *68. FM-model*, Delgado-Rizo 2017), (slide *69. SE-model*, Schon 2018). In (Herster 2018) hRNA-LL37 complexes take on the role of self-strengthening processes of neutrophil attraction into psoriatic skin, of chemokines and cytokine secretion and, basically, of netosis.

Recently carried out in vitro researches demonstrate netosis influence on T-lymphocytes and on their secretion of IL-17 (Lambert 2018). Blood cells were taken from healthy donors, but the main objective was to show that such influence is possible in psoriatic skin.

It is their chemokine receptors and certainly their agonists, which are responsible for neutrophil attraction to skin: chemokines and proteins with chemokine properties. Detailed information on chemokine receptors of neutrophils and their agonists is collected and analyzed below (Table 12**).**

Neutrophils are attracted into skin within subprocess LP1a along with other non-lymphocytic blood immunocytes. Subprocess LP1a begins within LPN3 - innate response, and after initiation of PLS-inflammation it is supported within vicious cycle B (slide *25. Local_processes_YN*).

Table 12. Chemokine receptors, chemokines and AMP positively or possibly involved in blood neutrophil traffic at psoriasis.

Chemokine receptors / Chemokine and AMP		CCR1	CCR2	CCR5	CCR6	CXCR1	CXCR2	CXCR4	FPRL1 (FPR2/ALX)
Common information			L	M		I	I, J	I, K, L	
New name	Old name								
CCL2	MCP-1	9.4	10.2 – B	7.5					
CCL3	MIP-1alpha	8.8 – A		8.9					
CCL5	RANTES	8.2		9.7					
CCL20	MIP-3alpha				8.5 - D				
CXCL1	Gro-alpha					6.4 – F	9.7 – F		
CXCL2	Gro-beta						9.1 – F		
CXCL3	Gro-gamma						9.2 – F		
CXCL5							9.0 - F		
CXCL8	IL-8					9.5 – F	9.5 – F		
CXCL12	SDF-1alpha							8.2 – G	
HBD-2			* - C		# - E				
HBD-3			* - C						
LL37									6.0 - H
FMLP									6.4

Notes (Table 12**).**

Similar information was earlier collected in (Kobayashi 2008, Soehnlein 2010, Blanchet 2012). This table includes all chemokines and AMP pertaining to traffic of blood neutrophils. Table cells specify the value of the maximum affinity between agonists according to DB IUPHAR. If value in IUPHAR is absent, but there is information on the ligand, the table cell contains #. If information on agonists is obtained from another source, the table cell contains *. The green color denotes receptor/chemokine interaction at homeostasis, pink – that during attraction of neutrophils into PLS. Latin capital letter is a cross-reference to one of the following notes:

A) CCR1 and CCL3 chemokine are responsible for attraction of CCR1+ phagocytes into psoriatic skin (Nickoloff 2007b). Neutrophils express CCR1 under the influence of GM-CSF (Kobayashi 2008).

B) CCR2 and CCL2 chemokine. CCL2 is secreted in normal skin (Ginhoux 2007), whereas in PLS it is secreted by KC much higher than normal (Vestergaard 2004). KC also secrete CCL2 in prepsoriatic skin (NLS) adjacent to plaque. In PLS and NLS secretion is concentrated in basal layer (Gillitzer 1993). Neu can express CCR2 (Rohrl 2010).

C) CCR2 and HBD-2, HBD-3. Antimicrobial proteins HBD-2 and HBD-3 are also active chemokines and contribute to attraction of all CCR2+ phagocytes from blood (Rohrl 2010). Increased HBD-2 level in blood flow correlates with psoriasis severity on PASI (Jansen 2009). AMP secretion (including HBD-2 and HBD-3) quantitatively and spatially depends on LP2 (initiating and aggravating process) and on the thickness of psoriatic plaque. This probably explains the differences in the results observed at mRNA HBD-2 psoriasis: double excess of the norm (Li 2004), 5000 times' excess (Gambichler 2008), and 20000 times' excess

(De Jongh 2005). In PLS-epidermis, most of HBD-2 is secreted by KC in the top spinous and granular layer, its concentration in the intercellular space reaches maximum in the cornual layer, where it fully envelops all KC (Huh 2002). In normal skin, HBD-2 presence is generally observed in basal layer (Poindexter 2005).

D) CCR6 and CCL20 chemokine. CCL20 is secreted in normal skin (Ginhoux 2007). Neutrophils express CCR6 under the influence of TNF-alpha (Kobayashi 2008).

E) CCR6 and antimicrobial protein HBD-2. HBD-2 in PLS is manifested intensely (Yang 1999), i.e. chemoattractant for CCR6+ phagocytes.

F) CXCR1, CXCR2 and CXCL1, CXCL8 chemokines. CXCL1 and CXCL8 are secreted by KC and are important for Neu attraction to PLS (Baker 2006c). AMP, CXCL1, CXCL2 and CXCL8 are found at extremely high level in psoriatic epidermis (Lin 2011). CXCL8 is constantly secreted in psoriatic skin (Gottlieb 2005), including by neutrophils (Duan 2001). CXCL1, CXCL2, CXCL3, CXCL5 are actively secreted in psoriatic skin, attracting CXCR2+ neutrophils (Guttman-Yassky 2011). CXCL1 is also ligand of CXCR1 receptor (Lowes 2013). In pro-inflammatory environment, neutrophils actively secrete CXCL8 chemokine which facilitates attraction of new neutrophils through their receptors of CXCR1 and CXCR2 (Soehnlein 2010). MMP9 protease increases chemokine activity of CXCL1 and CXCL8, thereby strengthening attraction of neutrophils. Similarly, MMP8 protease affects CXCL5 and CXCL8 (Soehnlein 2010).

G) CXCR4 and CXCL12 chemokine, a homeostatic pair. CXCL12 is continually secreted in normal skin (Bogunovic 2006, Urosevic 2005).

H) FPRL1 and LL37 protein. LL37 is ligand of FPRL1 – receptor which is expressed by blood phagocytes. Interaction of LL37 and FPRL1 supports chemotaxis of FPRL1+ phagocytes to inflammation sites (Sozzani 2005, De Yang 2000).

I) Neu well express CXCR2 receptor which determines their attraction to inflammation sites (Reddy 2010). Receptor expression changes in the course of aging: CXCR1 is expressed continually, CXCR4 expression increases whereas that of CXCR2 decreases (Rankin 2010). This is connected with the emergence of new Neu from bone marrow (CXCR2 - CXCL1) and with the return of senescent neutrophils into bone marrow (CXCR4 - CXCL12).

J) LTA impact on TLR2 receptor lowers CXCR2 receptor expression in neutrophils, which negatively influences blood neutrophil attraction to sites of secretion of CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8 chemokines (Reddy 2010).

K) LPS impact on neutrophils reduces CXCR4 expression. The degree of reduction depends on exposure time and LPS concentration; the reduction is two times at most (Kim 2007).

L) LPS influence (through TLR4) and PG (through TLR2) on neutrophils reduces CXCR4 expression and at the same time contributes to considerable CCR2 expression (Souto 2011, Shen 2017). This fact makes neutrophils fundamentally different from monocytes and dendritic cells, in which LPS influence causes reduction of CCR2 expression. Therefore blood neutrophils, being under chronic kPAMP-load, first pass into activated (prenetotic) state (Table 13). Then (if they do not undergo netosis in blood) they can be attracted to inflammation sites under the influence of CCL2 chemokine and HBD-2, HBD-3 proteins.

M) CCR5 is expressed only on apoptotic bodies of neutrophils. One of the aims of such expression is attracting and binding CCL2, CCL3, CCL5 chemokines, which decreases attraction of new neutrophils to inflammation site (Soehnlein 2010). Mass apoptosis of neutrophils is a sign of psoriatic plaque remission and, consequently, absence of necessity for attracting new neutrophils.

5.3.3. Netosis of neutrophils in skin (LP3a)

After being attracted into skin, neutrophils can undergo apoptosis, and apoptotic bodies are endocyted by other phagocytes (Greenlee-Wacker 2016, Soehnlein 2010). Neutrophils' tendency to apoptosis is, among other factors, determined by concentration of TNF-alpha, IFN-gamma and GM-CSF (van den Berg 2001, Soehnlein 2010). How exactly neutrophils in skin will terminate their existence (by apoptosis or netosis) depends on inflammatory process development. Predominant apoptosis and subsequent endocytosis (efferocytosis) of apoptosis products by other phagocytes, as a rule, means completion of inflammatory process (Soehnlein 2010, Schuster 2013, Malachowa 2016). Macrophages performing endocytosis of apoptosis products secrete anti-inflammatory cytokines TGF-beta, IL-10, and PGE-2 (Wang 2014a). Dendritic cells performing endocytosis of apoptosis products stop their maturing, i.e. their ability to transform into maDC and to present antigens (Schuster 2013). Chemokine receptor CCR5 is expressed only on apoptotic bodies of neutrophils. One of the aims of such expression is attraction and binding of chemokines CCL2, CCL3 and CCL5, which contributes to decrease of new neutrophil attraction to inflammation site and to the completion of inflammatory process (Soehnlein 2010).

Netosis, in its turn, occurs mainly in inflammatory environment and netotic products contribute to intensification of inflammatory process (Sangaletti 2012). It may be assumed that in psoriatic plaque, either stable or growing, neutrophils mainly end their existence by netosis. Only in case of plaque remission they end their existence mainly by apoptosis. Remission of psoriatic plaque and its transformation into normal skin leads to almost total neutrophil disappearance in it, since those which earlier arrived from blood have undergone netosis or apoptosis, whereas income of new neutrophils from blood nearly stops.

In active psoriatic plaque many neutrophils undergo netosis, and netotic products actively influence skin immune system (Lin 2011, Skrzeczynska 2012, Pinegin 2015, Hu 2016, slides *65. Net-skin-1* and *66. Net-skin-2*). Authors of two models of pathogenesis included netosis in the vicious cycle (slide *68. FM-model*, Delgado-Rizo 2017) and (slide *69. SE-model*, Schon 2018).

The most detailed analysis of the possible role of netosis in psoriatic inflammation is given in a survey (Pinegin 2015). Firstly, this is participation of netotic products (LL37, NE - neutrophil elastasa, etc.) in forming complexes with hDNA (influence PDC through TLR9) and RNA (influence DC through TLR7 and TLR8). Secondly, it is stimulation of pro-inflammatory cytokine secretion (IL-17, etc.) and, thirdly, it is formation of netotic products which can become autoantigens.

In (Herster 2018) hRNA-LL37 complexes have the role of self-amplification of the following processes: neutrophil attraction into psoriatic skin, secretion of chemokines and cytokines and, basically, netosis. For the first time it is demonstrated that netotic products contain hRNA. It is also demonstrated that LL37, which is, firstly, actively secreted by neutrophils and, secondly, contained in netotic products, forms complexes with hRNA lost during netosis. Besides, it is demonstrated that only these complexes (but not hRNA or LL37 separately) can influence neutrophils through endosomal receptor TLR8. Colocalization of NE (neutrophil elastasa), hRNA and LL37 in psoriatic skin is also shown. The authors of the work assume that netosis can pose a self-amplification factor of psoriatic plaque.

The role of netosis in pathogenesis of various diseases (systemic lupus erythematosus, rheumatoid arthritis, ANCA vasculitis, arterial and venous thrombosis, pulmonary fibrosis, psoriasis, gout) is systematized in (fig.1, fig.2, Mitsios 2017). It proposes a two-factor scheme of netosis role in pathogenesis of these diseases. The first factor (hit trigger) is the conditions under which proteins provoking a particular disease appear (are formed) in neutrophils; the second factor (hit trigger) is the conditions under which netosis takes place, accompanied by ejection of these proteins. The authors of this study suggested a new term "netopathy" to denote diseases in whose pathogenesis netosis plays the key role.

Within YN-model netosis of neutrophils in skin is conditioned, firstly, by chronic kPAMP-load on these neutrophils in blood before their attraction into psoriatic skin. This load makes some of them kPAMP-carriers and brings them into activated (prenetotic) state. As this occurs, part of neutrophils undergoes netosis directly in blood (Lin 2011, Hu 2016, slides *62. Net-blood-1*, *63. Net-blood-2* and *64. Net-blood-3*). Secondly, netosis in skin is determined by those conditions in which blood neutrophils (some of which are already in prenetotic state) find themselves in prepsoriatic and psoriatic skin (Table 13).

In prepsoriatic skin LP2 (initiating and aggravating process) and LPN3 (innate response) take place, and these processes, among other things, lead to secretion of CXCL8 and IL-1beta. In epidermis there are always bacteria (and bacterial products, specifically LPS and PG). Fungi presence is also possible and, if there is LP2(HPV), there are also HPV viruses. All these factors contribute to netosis.

In psoriatic skin, secretion of CXCL8 (particularly by neutrophils) and of IL-1beta is amplified, intensive secretion of IL-18 is added, and hRNA-LL37 complexes are formed. Microbiome concentration per unit of skin area also increases due to epidermis thickening. All this contributes to netosis.

During netosis of some neutrophils in skin, non-degraded proteins – kPAMP (specifically PG-Y) are ejected into extracellular space.

Netosis role in YN-model of pathogenesis fits into the scheme proposed in (Mitsios 2017) and, if YN-model proves to be right, psoriatic disease will be classified as netopathy.

Subprocess LP3a starts within LPN3 - innate response, and after initialization of PLS inflammation it is supported in vicious cycle B (slide *25. Local_processes_YN*).

Table 13. Real and presumed factors of netosis at psoriasis.

Factor	Sources	Systemic blood flow during SPPN	NLS - prepsoriatic skin	PLS - psoriatic skin
	Netosis is confirmed	Lin 2011, Hu 2016	?	Lin 2011, Hu 2016
Platelets	Pinegin 2015, Grayson 2016, Schon 2018, Papayannopoulos 2018	Yes (Clark 2007, Vorobjeva 2014, Garaeva 2007)	no	
ANCA (anti-neutrophil cytoplasmic antibody)	Grayson 2016, Hasler 2016, Delgado-Rizo 2017	?	?	?
ACPA (anti-citrullinated peptide antibody)	Hasler 2016, Delgado-Rizo 2017	?	?	?
CXCL8 (IL-8), self-amplification (?)	Grayson 2016, Hasler 2016, Ortmann 2018	yes	yes	
Complexes hRNA-LL37, self-amplification (?)	Herster 2018	?	?	yes
IL-18	Grayson 2016, Hasler 2016	yes	Yes, in epidermis during trauma (see LP2 (INJ), Peslyak 2012b)	yes, in epidermis
IL-1beta	Grayson 2016, Hasler 2016, Delgado-Rizo 2017	secretion by blood monocytes is possible	Yes, in the presence of HPV (see LP3 (HPV), Peslyak 2012b)	yes, it is actively secreted
Bacteria, fungi	Delgado-Rizo 2017, Kenny 2017, Papayannopoulos 2018	only at bacterial or fungal infection (sepsis)	Yes, especially in epidermis	
Viruses	Schonrich 2016 Delgado-Rizo 2017, Papayannopoulos 2018	only at virus infection	Yes, in epidermis in the presence of HPV (see LP2 (HPV), Peslyak 2012b) or of other virus skin infections	
Protozoan parasites	Grayson 2016, Hasler 2016, Papayannopoulos 2018	only at protozoan infection	no	
M1 protein (group A streptococci) + fibrinogen complex	Oehmcke 2009, Vorobjeva 2014	yes, at streptococcal infection (e.g. tonsillar infection)	no	
LPS	Hasler 2016, Pieterse 2016, Delgado-Rizo 2017, Lipp 2017, Papayannopoulos 2018, Ortmann 2018	yes (Garaeva 2007)	Yes, especially in epidermis	
PG	Hasler 2016, Alyami 2018	possible	Yes, especially in epidermis	
FMLP (N-Formylmethionyl-leucyl-phenylalanine) (synthesized by bacteria)	Hasler 2016, Lipp 2017	?	Yes, especially in epidermis	
Ionomycin and ionophore (synthesized by Streptomyces sp.)	Delgado-Rizo 2017, Kenny 2017	?	Yes, in case of using topical drugs which contain them	

5.3.4. Endocytosis of netotic products in skin (LP6a)

After netosis, NET (netotic products) are formed. They actively influence other cells, but are also endocyted by skin phagocytes (Soehnlein 2010, Schuster 2013, Wang 2014a, Grayson 2016). Endocytosis is effected by other neutrophils, macrophages, monocytes and dendritic cells of skin. When endocytosis is effected by monocytes Mo (having potential to be transformed into dendritic cells MoDC) and by dendritic cells DC, and if non-degraded antigens (particularly PG-Y) are retained in endocyted material, Mo-Y and DC-Y are formed (LP6a).

Next, transformation of Mo-Y into MoDC-Y (LP6b), and then transformation of DC-Y and MoDC-Y into mature dendritic cells maDC-Y (LP6c) is possible. As a result, presentation of Y-antigens to effector lymphocytes TL-Y becomes possible (slides *25. Local_processes_YN* and *26. YN-model*).

Phagocytes (monocytes and dendritic cells), which endocyted apoptosis products of neutrophils, can entrap even live intracellular bacteria lost by neutrophils (Schuster 2013, McCracken 2014, Karaji 2017) and thus to become infected.

Interaction of dendritic cells with apoptosis products has been studied for a long time. It has been demonstrated that they are able to effect processing and presenting antigens which were endocyted together with apoptosis products (Schuster 2013, Nauseef 2013).

Comparison of these processes has been made on the example of ANCA vasculites, when autoantibodies are formed to proteins PR3 or MPO, which are products of apoptosis and netosis of neutrophils. It has been demonstrated that these proteins to a much greater extent retain their autoantigenic properties during netosis. Formation of autoantibodies takes place with the active participation of dendritic cells which endocyte netotic products and then process and present autoantigens contained in them (Sangaletti 2012, video of endocytosis).

Taking into account the above-mentioned information, endocytosis of non-degraded bacterial products lost during netosis in psoriatic skin by monocytes and dendritic cells seems quite possible. And subsequent processing and presentation of Y-antigens contained in them are their direct responsibility (monocytes Mo-Y perform it after transformation into dendritic cells MoDC-Y).

Subprocess LP6a begins owing to LPN3 (innate response), and after initialization of PLS inflammation is supported in vicious cycle B (slides *25. Local_processes_YN* and *26. YN-model*).

5.3.5. Roles of neutrophils

All information on known and presumed roles of neutrophils at psoriatic disease is collected and expanded below (Table 14).

Table 14. Roles of neutrophils at psoriatic disease: known and presumed.

	Roles	Locali-zation	Status	Notes
1	Endocytosis of any non-host products contained in blood	Systemic blood flow	proved	Well-known
2	Netosis, ejection of netotic products into extracellular space.	Systemic blood flow	proved	Lin 2011, Skrzeczynska 2012, Hu 2016. slides *62. Net-blood-1*, *63. Net-blood-2*, *64. Net-blood-3*
3	Attraction from systemic blood flow into prepsoriatic and psoriatic skin		proved	Van de Kerkhof 2007, Christophers 2014, Gilliet 2008 (GL-model), Perera 2012 (N-model), Peslyak 2012b (Y-model), YN-model (section 5.2). slides *59. Neutrophils* and *60. Neutrophils-Munro*
4	Netosis, ejection of netotic products into extracellular space.	Psoriatic skin	proved	Lin 2011 (KB-scheme), Skrzeczynska 2012, Lowes 2014, Hu 2016, Schon 2017, Schon 2018 (SE-model), YN-model (section 5.2). slides *65. Net-skin-1* and *66. Net-skin-2*
4.1	LL37 - active secretion, as well as ejection during netosis	Psoriatic skin	proved	Only secretion: Gilliet 2008, Ganguly 2009 (GL-model), Guttman-Yassky 2011 (GK-model), Perera 2012 (N-model), Peslyak 2012b (Y-model). Lowes 2014, Hawkes 2017 (GKH-model), Secretion and ejection during netosis: Lin 2011 (KB-scheme), Pinegin 2015, Delgado-Rizo 2017 (FM-model), Schon 2018 (SE-model), YN-model (section 5.2).
4.2	Active secretion of IL-17			Lin 2011 (KB-scheme), Pinegin 2015, Schon 2018 (SE-model), YN-model (section 5.2).
4.3	Active secretion of TNF-alpha, IL-22			Schon 2018 (SE-model), YN-model (section 5.2).
4.4	Ejection of hDNA during netosis			Lin 2011 (KB-scheme), Pinegin 2015, Delgado-Rizo 2017 (FM-model), YN-model (section 5.2).
4.5	Ejection of hRNA during netosis			Herster 2018
4.6	Ejection of MPO (myeloperoxidase) during netosis			Schon 2018 (SE-model).
5	Impact on plasmacytoid dendritic cells PDC, contributing to active secretion of IFN-alpha, through hDNA-LL37 complexes.	Psoriatic derma	proved	hDNA source - damaged keratinocytes: Gilliet 2008, Ganguly 2009 (GL-model), Tonel 2009 (TC-model), Guttman-Yassky 2011 (GK-model), Perera 2012 (N-model), Peslyak 2012b (Y-model). hDNA source - netotic products: Lin 2011 (KB-scheme), Skrzeczynska 2012, Delgado-Rizo 2017 (FM-model) Schon 2018 (SE-model). Panda 2017 (survey of PDC properties), YN-model (section 5.2).
6	Impact on dendritic cells DC, contributing to their maturing, through hRNA-LL37 complexes.	Psoriatic derma	proved	hRNA source - damaged keratinocytes: Gilliet 2008, Ganguly 2009 (GL-model). hRNA source - netotic products: Herster 2018
7	Netotic products may contain non-degraded non-host products, earlier endocyted in blood (particularly Y-antigen).	Psoriatic skin	possible	Hypothesis H12. YN-model (section 5.2).
8	Neutrophils Neu-Y can independently present Y-antigen to specific TL-Y-lymphocytes.	Psoriatic skin	possible	Davey 2014, Lin 2017. Under the influence of GM-CSF, IFN-gamma, IL-4 and TNF-alpha, as well as when interacting with memory CD4+ T-lymphocytes, neutrophils can perform the functions of antigen-presenting cells.

Table 15. Models of psoriasis pathogenesis and neutrophils.

Patho-genesis model	Sources	Neutrophils in systemic blood flow	Neutrophils in psoriatic skin	hDNA-LL37 complexes affecting PDC and/or hRNA-LL37 affecting DC	Netosis in psoriatic skin	Notes
BF-model	Baker 2006b	no	no	no	no	Systemic. Presumed antigen - peptidoglycan fragment.
N-model	Perera 2012	no	yes	LL37 and hDNA from damaged keratinocytes	no	Unknown antigen (keratin?).
GK-model	Guttman-Yassky 2011	no	LL37 secretion	hDNA from damaged keratinocytes	no	Unknown antigen (keratin?). Vicious cycle.
TC-model	Tonel 2009	no	no	LL37 and hDNA from damaged keratinocytes	no	
GL-model	Gilliet 2008, Ganguly 2009	no	LL37 secretion	hDNA and hRNA from damaged keratinocytes	no	Vicious cycle.
Y-model	Peslyak 2012a, Peslyak 2012b	Bone marrow transformation Mo and DC with apoptotic neutrophil participation.	LL37 secretion	hDNA and hRNA from damaged keratinocytes	no	Systemic. Presumed Y-antigen - peptidoglycan fragment. Includes the proved links of models BF, N, GK, GL and TC. Compatible with YN-model. Two vicious cycles.
KB-scheme	Lin 2011	no	LL37 and IL-17 secretion	hDNA from netosis	Yes. Ejection of hDNA, LL37.	Vicious cycle (includes netosis). Netosis is taken into account for the first time.
FM-model	Delgado-Rizo 2017	no	LL37 secretion	hDNA from netosis	Yes. Ejection of hDNA, LL37.	Unknown antigen (only in the scheme). Vicious cycle (includes netosis). Extension of KB-scheme.
SE-model	Schon 2018	no	Secretion of LL37, IL-17, TNF-alpha, IL-22	Not present (mentioned in the text but absent on schemes)	Yes. Ejection of LL37, IL-17, MPO	Potential antigens - keratin 17, streptococcal protein M1, LL37, ADAMTSL5. Vicious cycle.
GKH-model	Lowes 2014, Hawkes 2017	no	LL37 secretion	LL37, hDNA and hRNA from damaged keratinocytes	no	Specification of GK-model. Potential antigens - keratins, streptococcal proteins, ADAMTSL5. Vicious cycle.
BMM-model	Benhadou 2018	no	no	Not present (mentioned in the text but absent on schemes)	no	Potential antigens - LL37, ADAMTSL5. Vicious cycle.
YN-model	section 5.2	Endocytosis of kPAMP, including that of Y-antigen.	Secretion of LL37, IL-17, HBD-2, AMP	LL37, hDNA and hRNA at chronicity, primarily from netosis	Yes. Ejection of hDNA, hRNA, LL37. Ejection of non-degraded kPAMP, including that of Y-antigen.	Systemic. Presumed Y-antigen - peptidoglycan fragment. Includes the proved links of BF, N, GK, GL, TC, FM, SE models. Compatible with Y-model. Two vicious cycles: the first one includes netosis.

5.4. Bacterial species presumed psoriagenic

SIBO is characteristic of psoriatic patients (Peslyak 2012, Gumayunova 2009a, Gumayunova 2009b, Gumayunova 2009c). Small intestine microbiome is believed to contain abundant bacteria called psoriagenic (Peslyak 2012a, Peslyak 2012b). These are bacteria having peptidoglycan similar to Str.pyogenes, i.e. containing (L-Ala)-(L-Ala) and/or (L-Ser)-(L-Ala) interpeptide bridges. Formation of these bridges in peptidoglycan is conditioned by the existence of enzymes of murM and murN type (slide *17. PG_PsB-2*).

murM is an enzyme, providing serine/alanine addition (the first amino acid starting with Lys) when forming an interpeptide bridge at peptidoglycan. In the absence of this enzyme, there will be hardly any

bridges. What exactly is added (serine or alanine) depends on the allele of murM-gene (Filipe 2001, Fiser 2003).

murN is an enzyme, providing alanine addition (the second amino acid starting with Lys) when forming an interpeptide bridge at peptidoglycan. In the absence of this enzyme, the bridge will be one amino acid in length.

DB KEGG makes it possible to determine all (listed in it) bacterial strains which have genes providing secretion of both enzymes, i.e. both of murM type and murN type. Formation of interpeptide bridges in different bacteria is provided by various murMN-genes. Slide *18. PG_PsB-3* catalogs species of such bacteria. All strains of each of these species have (L-Ala)-(L-Ala) and/or (L-Ser)-(L-Ala) interpeptide bridges, i.e. their peptidoglycan is similar to Str.pyogenes peptidoglycan. All these species are presumed psoriagenic (labeled PsB).

If it is proved that Enterococcus faecalis is psoriagenic, it will be possible to draw a conclusion that only those strains are psoriagenic in which murM-gene allele provides preferential addition of alanine, i.e. formation of (L-Ala)-(L-Ala) bridge. As a consequence, the list of peptides – potential Y-antigens – will be abridged (section 5.5). It will only include those which contain (L-Ala)-(L-Ala) on epitopic site. Such a conclusion can be drawn as all known strains of Enterococcus faecalis have only interpeptide bridge (L-Ala)-(L-Ala).

5.5. Peptides – potential Y-antigens

As not the whole antigen but only its central part, called epitope, is presented, the concept of Y-antigen implies a certain set of peptides having common epitopes (i.e. almost identical central parts).

During psoriatic inflammation preferential presentation of unknown Y-antigen by mature dendritic cells through MHC II takes place. Thus, they present Y-antigen to specific CD4+TL.

At the initial stage of psoriatic inflammation and further (but to a lesser extent), Y-antigen cross-presentation by mature dendritic cells through MHC I also takes place. Thus, they present Y-antigen to specific CD8+TL.

MHC characteristics determine the structure and size of presented peptides. The structure must be close to linear, while peptide length is to be within the range of 8-11 amino acids (for MHC I) and 11-17 amino acids (for MHC II). Branched molecules and big size molecules before presentation are processed so that these requirements are fulfilled, but at the same time so that characteristic epitope is retained.

Slide *19. PG_PsB-4* shows muropeptides and peptides formed at peptidoglycan degradation of Str.pneumonia (one of the species presumed psoriagenic). Muropeptides 20, 21, 23 and 25 affected by NAMLAA amidase (EC 3.5.1.28) form linear peptides 5 and 6a (numbering by Bui 2012). These peptides contain epitope IB-Y and have length of 9 amino acids, which enables them to be presented through MHC I. MDP, being adjuvant, ensures processing and presentation intensity. Linear peptides 5 and 6a are potential Y-antigens.

Slide *20. PG_PsB-5* shows B15-3 peptide (the name is conventional) which, affected by NAMLAA amidase (EC 3.5.1.28), is formed from muropeptides 33, 34 and 35 (numbering by Bui 2012). Depending on the number of cuts made by GDGDA endopeptidase (the name is conventional, EC 3.4.14.13), B15-3 peptide gives peptides from 13 amino acids (three options, one of them being B13-2), from 11 (three options) or from 9 amino acids.

B15-3 peptide (and its analogs), as well as some of its derivatives (from not less than 11 amino acids), are potential Y-antigens presented through MHC II. In this case, one or both IB-Y bridges will act as epitopes. MDP, being adjuvant, ensures processing and presentation intensity.

Slide *21. PG_PsB-6* shows peptide IX (similar to peptides VII and VIII) which, affected by NAMLAA amidase (EC 3.5.1.28), are formed from muropeptides 36 and 37 (numbering of peptides and muropeptides follows Bui 2012 and Filipe 2000). Depending on the number of cuts made by GDGDA endopeptidase (EC 3.4.14.13), peptide IX gives peptides either from 15 amino acids (three options), from 13 amino acids (three options) or linear Y-B11 peptide from 11 amino acids.

Peptide IX (and its analogs - peptides VII and VIII), as well as some of their derivatives (from not less than 11 amino acids) are potential Y-antigens, presented through MHC II. In this case, one or both central IB-Y bridges will act as epitopes. MDP, being adjuvant, ensures processing and presentation intensity.

The first estimate is made on the basis of MDP percentage in peptidoglycan (Sekine 1985), presumed density of interpeptide bridges in 50% (Vollmer 2010) and the proportion between MDP content and B13-2 (or Y-B11) in peptidoglycan.

The second estimate for B13-2 is made on the basis of summary content of muropeptides 33, 34 and 35 (~ 1.5%) in Str.pneumonia peptidoglycan strain R6 and on the basis of the proportion of B13-2 peptide

weight and the average weight of these muropeptides (Bui 2012). The second estimate for Y-B11 is made on the basis of summary content of muropeptides 36 and 37 (~ 1.3%) in Str.pneumonia peptidoglycan strain R6 and on the basis of the proportion of Y-B11 peptide weight and the average weight of these muropeptides (Bui 2012).

Table 16. Assessment of peptide weight.

Peptide - potential Y-antigen	Assessment	pcs/cell	g/cell	% of cell weight
B13-2	1	~10^6	$2.1*10^{-15}$	0.21%
	2	~$6*10^5$	$1.2*10^{-15}$	0.12%
Y-B11	1	~$5.3*10^5$	$8.5*10^{-16}$	0.08%
	2	~$5*10^5$	$8.0*10^{-16}$	0.08%

All estimates have yielded close results and on their basis it will be possible to estimate concentration of B13-2 and Y-B11 in psoriatic skin. This can be done by total bacDNA concentration of species (strains) of bacteria, presumed psoriagenic, in MPSN – a subset of non-resident origin in MPS (Supplement S4.2, section 5.12). Note that density and composition of interpeptide bridges of these bacteria must be taken into account. Such an assessment method is appropriate if concentration of bacterial products, such as bacDNA, muropeptides and peptides derivative of peptidoglycan, at income in blood flow, endocytosis (binding) and degradation by phagocytes and transportation of non-degraded parts into psoriatic skin, change proportionately.

5.6. Determining PAMP concentration in blood

Within task T2 it is suggested to carry out several standard biochemical tests determining PAMP-nemia level (tasks T2.4 and T2.5). The biomaterial is whole blood or whole blood after cell destruction. It is a standard test to determine LPS concentration: LAL-test (Limulus Amebocyte Lysate with suppression of (1,3)-beta-D-glucan influence on the result). The test will enable us to determine Qlpsb - LPS concentration by a standard proven method and to compare it with Qminus - total bacDNA concentration of Gram(-) species (task T3.5).

> Total LPS-level for 16 patients with moderate psoriasis (average PASI = 16.5) was 7.2 Eu/ml. Total LPS-level for 30 patients with serious psoriasis (average PASI = 24) was 35.8 Eu/ml. Total LPS-level for patients with erythrodermic and exudative psoriasis was from 1000 to 2800 Eu/ml. Total LPS-level in control group (112 healthy persons) was about 0.1 Eu/ml. (Garaeva 2007). LPS-level in blood was determined using a modified LAL test.

An alternative method of assessing LPS concentration in blood is EAA-test (Spectral Medical Inc.) (Ishihata 2013). This method is more accurate than LAL-test at concentration of LPS < 0.25 Eu/ml, it has been approved by FDA since 2003 and is used to assess the risk of sepsis development. Assessment of LPS concentration in psoriatic patients' blood by EAA has never been carried out.

Besides, it is determining Qsum – total concentration of PG and (1,3)-beta-D-glucan by SLP-test (Wako Pure Chemical Industries, Ltd.) and Qglu - concentration of (1,3)-beta-D-glucan in biomaterial by one of the standard tests. Let us enumerate them: Fungitell (Cape Cod, Inc.), Endosafe-PTS glucan assay (Charles River Laboratories International, Inc.), Fungus (1-3)-β-D-Glucan Assay (Dynamiker Biotechnology Co., Ltd.) or Goldstream Fungus (1-3)-β-D-glucan (Era Biology Group) (task T2.5) (Barreto-Bergter 2014, Wright 2011). This will enable us to determine Qpgb (through Qsum and Qglu) - PG concentration by standard proven methods and to compare it with Qplus - total bacDNA concentration of Gram+ species (task T3.6). Assessment of PG concentration in psoriatic patients' blood has never been carried out.

It will also be possible to compare Qglu with total concentration of fungal DNA. The results of these tests will enable us to expand and specify information obtained by WMS-tests as well as to determine their correlation with PD severity. Assessment of (1,3)-beta-D-glucan concentration in psoriatic patients' blood has never been carried out.

> PG concentration in blood is normal (control group of 14 people) on average - 20 pg/ml (from 0 to 90), PG concentration in blood in patients with sepsis from 50 pg/ml (Fitting 2012) to 190 ng/ml (Kobayashi 2000).

To determine Qpgb, instead of using SLP-test combined with one of the standard tests for (1,3)-beta-D-glucan we can apply one of Human Peptidoglycan Elisa kits. Let us enumerate them: CEO769Ge (Cloud Clone), MBS751887 (competitive or sandwich polyclonal) (MyBiosource), NB-E11251 (Novateinbio), 22749 (Bmassay), AMS.E01P0182 (AMS Biotechnology), BC-EH102570 (Biocodon Techonologies), RS02E1322 (BioTecNika Labs), E028552 (Biobool). When choosing Elisa-set, it is important to consider its versatility, i.e. if the set can equally well determine peptidoglycan concentration of any Gram(+) genera of bacteria and has

a minimal dependence on the presence of LPS or (1,3)-beta-D-glucan. Using such Elisa-set will become an optimal solution.

In addition to task T2.4, it is suggested to estimate Qlpsm - total bacDNA concentration of bacteria with TLR4-active LPS in whole blood (task T3.5). This can be done by determining simultaneous presence of KO (KEGG orthologs) of bacterial genes of LpxL (K02517) and LpxM (K02560) type in genomes of Gram(-) species. These genes are responsible for LPS biosynthesis in Gram(-) bacteria, which have maximum TLR4-activity (Maeshima 2013, Gnauck 2016b). As a result, correlation between Qlpsm and PD severity can be established (Fukui 2016).

With insufficient coverage – determining Qlpsm through representation of genera nearly all species of which have genes of LpxM and LpxL.

Essential difference in the ability to influence netosis of LPS from different species and strains of bacteria has been discovered recently (Pieterse 2016). It may be assumed that it is also connected with their different TLR4-activity.

In addition to tasks T3.6 and T3.7 (Table 8), it is possible to determine and compare total concentration KO (KEGG orthologs) of bacterial genes of murM (K12554) and murN (K05363) type contained in genomes of Gram+ species. This can be done in the way it was performed for patients with atherosclerosis, when excess over norm of KO (KEGG orthologs), responsible for peptidoglycan biosynthesis, was discovered in fecal metagenome (Karlsson 2012). In such a way, we can, firstly, expand and modify the list of bacteria species presumed psoriagenic (slide *18. PG_PsB-3*). Secondly, we can expand the information on the correlation between Qpsb and PD severity (task T3.7).

5.7. Contamination control. Samples of NTC.

It is important to minimize contamination of blood samples by skin microbiome during venipuncture. If elementary precautionary measures are not taken, e.g. concentration of bacDNA Propionibacterium acnes (and other skin commensals) can prove to be of the same order as total bacDNA concentration in plasma (Grumaz 2016, Horiba 2018, Kimer 2018). One of such measures is collecting blood samples for metagenomic research into the last test tube (not earlier than the third one) from those consecutively attached to the needle (samples from initial test tubes can be used for biochemical research).

During project implementation, special attention will be given to controlling contamination level and taking measures to minimize it. To achieve this, along with blood (skin) samples, several samples of NTC (no template control) are formed (Grumaz 2016, Païssé 2016, Kim 2017, Kong 2017, Puri 2018, Li 2018, Qian 2018). Instead of blood (skin) samples, culture of sterile human cells and, if situation requires, biologically clear water (e.g. RT-PCR Grade Water) is placed in test tubes (Kowarsky 2017). Samples of NTC are to be similar (in their amount, DNA concentration, etc.) to blood (skin) samples.

Samples of NTC are formed on the day of blood (skin) sampling, in the same room where it is carried out. This is done by the same employee who carries out blood (skin) sampling. If blood (skin) sampling is carried out within a few days, samples of NTC are formed on each of these days. Subsequent storage, transportation and processing of samples of NTC should repeat the procedure of processing blood (skin) samples with the greatest possible accuracy. The total number of samples of NTC, the order of their formation and processing will be established during the pilot stage of the project.

During protocol implementation (section 5.10), contamination level will be checked a few times. After particular steps of the protocol (n denotes step number), AB(n, j) – total bacDNA concentration in blood samples and TB (n, j) – in samples of NTC will be determined, and then contamination level will be calculated using formula (1).

(1) $ContB(n)=TBM(n)/ABM(n)$,

where ABM(n) stands for average bacDNA concentration in all blood samples after step B(n), calculated using formula (2), and TBM(n) is average bacDNA concentration in all NTC after step B(n) calculated by formula (3).

(2) $ABM(n) = (1/KB)*\sum AB(n,j)$, $j=1,\ldots,KB$, n - step number in the protocol, KB - number of blood samples.

(3) $TBM(n) = (1/KTB)*\sum TB(n,j)$, $j=1,\ldots,KTB$. n - step number in the protocol, KTB - number of samples of NTC studied together with blood samples.

It is probably more correct to calculate TBM(n) by formula (4), i.e. to use not the average, but the maximum. This will be decided during the pilot stage.

(4) $TBM(n) = \max(TB(n,j))$, $j=1,\ldots,KTB$. n - step number in the protocol, KTB - number of samples of NTC studied together with blood samples.

As a result of each check, contamination level ContB(n) must not exceed 0.05% (this will be determined more precisely during the pilot stage). It is achievable since such a level or even a lower one can be achieved (Table 5): Paisse 2016 (0.048% - normal, good), Li 2018 (0.003% - very low, excellent), Qian 2018 (0.14% - high, unsatisfactory).

It is essential, as it is determined by the project objective: studying bacDNA concentration of PsB – species presumed psoriagenic (of each of them separately and of the total). According to (Paisse 2016), total PsB representation does not exceed 2.25% (this is a rough estimate as 16S-test was used). According to (Grumaz 2016), total PsB representation constitutes 5.75%, whereas representation of eight main PsB lies in the range of 0.3% to 1.2% (slide *36. Blood-Germany-2*). To ensure that representation values for the main PsB species are reliable for each PP and HP, contamination level is to be considerably lower than expected values, i.e. not higher than 0.05%.

Full information on contamination composition (NTC metagenome) will be obtained according to WMS-test results. This will enable us to exclude the results on all nhDNA whose concentration in blood samples proves not higher than maximum total nhDNA concentration in NTC samples.

An alternative approach presupposes excluding results only for those nhDNA whose concentration in blood samples proves not higher than maximum concentration of the same nhDNA in samples of NTC.

It is also possible to exclude results on nhDNA which are presumed to be the most probable contaminants (Horiba 2018, Kimer 2018). This approach makes sense if control of contamination using samples of NTC was insufficient or not carried out at all.

This approach makes sense if control of contamination using NTC samples was insufficient or not carried out at all.

Within the project, all preventive measures will be taken to reduce contamination, so that it should not materially affect the results. Specifically, all work with samples is to take place in a laboratory of at least "biosafety class II" or higher.

If the average contamination level exceeds admissible (whose value will be recorded in specification), its cause must be established and eliminated, while protocol steps during which it occurred are to be repeated.

5.8. hDNA elimination from DNA-samples

Tasks T3.5 and T3.7 cannot be fulfilled by 16S-test. The reason for it is considerably lower capacity of 16S-test compared to WMS-test (Supplement S1, section 5.12). Consequently, within the project, we are to perform WMS-tests. WMS-test used to be applied to identify nhDNA only in blood plasma (Grumaz 2016, Gyarmati 2016, Long 2016), with no preliminary hDNA elimination carried out. However, preliminary elimination was successfully applied in other studies (Archer 2010, Fitting 2012, Opota 2015, Song 2014, Yigit 2016, Marotz 2018) and is going to be performed within the project.

Preliminary hDNA elimination method realized in NebNext Microbiome DNA Enrichment kit (henceforth NME) is described and studied in (Feehery 2013, Yigit 2016). Enrichment is achieved by hDNA binding and its subsequent elimination (in which case DNA of some bacteria species can also be partly bound and eliminated) (slides *78. NME-1* and *80. NME-2*). In (Feehery 2013) enrichment of several artificial biomaterials (mixture of hDNA and bacDNA of E.coli in different proportions) as well as of two saliva samples and one blood sample was studied. For saliva samples, the enrichment was successful. The percentage of hDNA fell by 20 times approximately (94-96% of hDNA was eliminated), and the percentage of mapped bacDNA reads rose by 8 times on average (slide *81. NME-3*). Mapping was carried out on reference database of oral microbiome (HOMD). Notably, bacDNA representations in enriched samples fully corresponded to the initial ones (except for bacDNA found only in the enriched sample and for species with low representation) (chart for R2, fig. 6, Feehery 2013). Average Pearson correlation coefficients between species representation (before and after NME use) are given below (Table 17).

Blood sample enrichment of (buffy coat) gave dubious result (according to Feehery 2013) due to a very small absolute bacDNA quantity as well as to possible sample contamination. Up to now, there have been no other papers about research where NME is applied to DNA-samples of whole blood.

In (Yigit 2016) contains a detailed protocol of NME performance.

In (Marotz 2018) the authors suggest their own method of preliminary elimination, similar to the one realized in Molysis kit. Molysis and (Marotz 2018) methods are not suitable for the project objectives as they are meant for sepsis diagnostics - identifying metagenome of whole non-host cells outside blood cells. These methods are based on preliminary destruction of blood cells and subsequent full degradation and elimination of all DNA in them (when this occurs, all nhDNA which was inside blood phagocytes is also lost). Afterwards, mainly whole non-host cells are left in the sample (as their walls are stronger than those of blood cells); they are destroyed, and nhDNA is extracted.

For the project objectives, though, the work (Marotz 2018) is important primarily by the fact that it compares several various methods of hDNA elimination on saliva samples, including NME. In this study, saliva samples from 8 HP were taken, and for each sample three WMS-tests were carried out, before and

after NME use. Some of the results (concerning NME) are given in slide *82. NME-4*. Unfortunately, the researchers did not perform obligatory sample preparation before NME use, namely they did not remove DNA fragments smaller than 15 KB (which is highly recommended by NME manual). This led to inefficient NME use (DNA concentration fell by almost 10 times whereas hDNA representation did not change: before – 89.3% and after – 90.8%). The authors gave a detailed account of it in (Discussion, Marotz 2018).

Saliva metagenomes were obtained by consecutive processing of reads (Sequencing data analysis, Marotz 2018). Notably, reads algorithmically filtered from hDNA were published in European Nucleotide Archive DB (ENA, PRJEB24090). Thereby we acquired an opportunity to estimate NME influence on species representation. Online usegalaxy.org resource was used for the purpose. Using it, we consecutively applied "Kraken assign taxonomic labels to sequencing reads" on reference DB "bacteria" and then "Kraken-filter classification by confidence score" with 0.5 parameter to 48 read sets (8 patients x 3 aliquots per sample x 2 (before and after NME)). For comparison (before and after), we chose only those reads which were mapped on species taxon rank or below (for calculating, the option "Summation of lower ranks into higher ranks" was selected).

Average Pearson correlation coefficients between representation of several subsets of species before and after NME use are gathered in Table 17. As is clear from the table, the smallest correlation is observed for minor species (whose representation in initial samples does not exceed 1%). However, correlation becomes high with representation increase. We assume that one of the major reasons for it is insufficient coverage of minor species in initial samples. In samples of patient "A" (before enrichment), for instance, major species of Veillonella parvula, Prevotella melaninogenica and Haemophilus parainfluenzae were represented on average by 25.1%, 24.0% and 22.3% respectively, so their coverage (taking into account genome size) averaged 3.4. For species whose representation was less than 1%, however, coverage did not exceed 0.15. For the same species after enrichment, coverage for the same patient's samples was even lower (1.47 and 0.06 respectively), which is connected with the above mentioned absence of sample preparation before NME use.

In case of NME use in our project, it is necessary to ensure both the exact observance of sample preparation requirements and higher coverage for species with representation of 0.1% to 1% (by increasing the number of reads for one sample).

Table 17. Correlation between representation before and after hDNA elimination.

Subset of bacteria, with representation (in samples before hDNA elimination)	Pearson correlation coefficient between representation before and after hDNA elimination		
	Saliva, NME, species, WMS-test, n=2, Feehery 2013	Saliva, NME, species, WMS-test, n=8, 3 aliquots per sample, Marotz 2018	Submucosal small intestine biopsies, LOOXSTER, genera, 16S-test, n=4, Glassing 2015
All	0.960 ***	0.902 ***	0.826 **
> 0.1%	0.953 ***	0.889 **	0.789 **
> 1%	0.923 ***	0.820 **	0.721 **
From 0.1% to 10%	0.925 ***	0.858 **	0.632 *
From 0.1% to 5%	0.863 **	0.767 **	0.693 *
From 0.1% to 1%	0.754 **	0.609 *	0.536 *

Chaddock scale correlation: *** - very high, ** - high, * - noticeable.
Calculation of coefficients is made by the authors of this book.

There is an important question, the answer to which is to be received during the pilot stage (task T1.4). It is necessary to check what percentage of nhDNA will be lost while eliminating DNA fragments with the size of < 15KB (obligatory DNA sample preparation for NME). Information on fragmentary nhDNA distribution in HP whole blood is absent at the moment. During the pilot stage it is expected to obtain this information for bacDNA in whole blood of HP and PP (task T1.4.1).

Firstly, the main source of bacDNA fragment income is small intestine microbiome and, consequently, their size is limited by characteristics of small intestine permeability; secondly, bacDNA fragments first appear in blood flow where they become exposed to degradation by enzymes; and thirdly, they are endocyted by blood phagocytes, in which degradation continues.

Getting into blood flow, bacDNA is exposed to all those influences which extracellular hDNA experiences. Normal extracellular hDNA has low-molecular fragmentation (from 140 to 250 bp), which, among other things, is dictated by the degrading effect of blood enzymes (Bryzgunova 2015, Aucamp 2018, Suzuki 2008).

If the median of fragmentary bacDNA distribution proves to be noticeably smaller than 15 KB, with DNA fragments of the size <15 KB eliminated, a considerable proportion of initial bacDNA will be lost. This percentage will be so considerable that NME use for the remaining DNA fraction (> 15 KB) will not ensure effective enrichment compared to the initial DNA sample.

Moreover, it can turn out that, instead of using a large (> 15 KB) DNA fraction and its processing by NME, it is reasonable to use a small fraction without NME use. And the optimum borderline between small and large fractions can differ from 15 KB (this will become clear following the results of solving task T1.4.1).

Really, suppose

(5) D = DL + DH, where

D is the amount of all DNA subject to division (by agarose gel electrophoresis) into two fractions: small and large; DL is DNA amount in the small fraction and DH – in the large fraction.

(6) DB = DBL + DBH, where

DB is the amount of bacDNA subject to division into two fractions: small and large; DBL is bacDNA amount in the small fraction and DBH – in the large fraction.

D and DB are determined before fractionation, DH and DBH – after separation of the large fraction.

Suppose ko is effectiveness ratio of enrichment by NME. If DL amount is sufficient for library formation and inequality (7) is carried out, NME use is inexpedient, and to prepare the library we have to use DL – the small fraction.

(7) DBL / DL > DBH / (DBH + ko*(DH-DBH))

For example, suppose DL = 0.1*D; DH = 0.9*D; DB = 0.01*D; DBL=DBH=0.5*B; ko = 0.2; then, by substitution in (7) we will receive 0.05 on the left and 0.027 on the right, and if DL amount is sufficient for library preparation, NME use is inexpedient. It is necessary to extract all DNA from the lower part of agarose gel (the small fraction), and it is this very fraction that should be used for further research.

However, if ko = 0.02 (with the other parameters being the same), in (7) we will have the same value of 0.05 on the left and ~ 0.22 on the right, so NME use will be reasonable. Note that according to the results of correct NME use (Feehery 2013), coefficient ko does not exceed 0.02, but it has not yet been substantiated by other studies.

The use of the small fraction as of the enriched one depends on the characteristics of DNA isolation kit, primarily on fragmentary distribution of all DNA (provided by the kit manufacturer) as well as on (unknown) bacDNA distribution. So, if it turned out that the median of bacDNA distribution is considerably smaller than the median of all DNA distribution, enrichment would be possible by separating the small fraction (the division line between medians is determined empirically and can differ from 15 KB). The quality of such enrichment certainly depends not only on the arrangement of medians, but also on each of the two distributions in general.

Let us consider distribution of all DNA fragmentation for Gentra Pure Gene Blood kit (Qiagen) (Malentacchi 2015, slide *79. NME-1a*, A). DNA integrity (fragmentary DNA distribution) is estimated (according to T0) approximately as (median 70 KB, deviation 10 KB). Below you can find three charts (B1, B2 and B3) of possible bacDNA distribution contained in this DNA. For descriptive reasons, total bacDNA volume constitutes 1% of all DNA (the known results vary from 0.03% to 0.2%).

- (B1) BacDNA distribution with 70 KB median: there is no point in eliminating fractions smaller than 15 KB in size. NME use is obligatory.
- (B2) BacDNA distribution with 50 KB median: enrichment can be achieved by eliminating fractions larger than 55 KB in size; NME use is questionable.
- (B3) BacDNA distribution with 10 KB median: a fraction smaller than 15 KB in size must not be eliminated, but we can eliminate fractions larger than 30 KB in size; afterwards NME must not be applied!

Note that there are kits and standard methods which preserve the integrity of DNA isolated from whole blood to a greater extent (median up to 130 KB). Following the results of solving task T1.4.1, we can choose the most suitable kit (or standard method) within which the median of bacDNA distribution will be most remote (probably less) from the median of all DNA distribution, which will enable us to conduct hDNA elimination most effectively, and possibly without NME use.

LOOXSTER® Enrichment Kit uses PureProve enrichment method based on binding an essential part of bacterial and fungal DNA and subsequent elimination of all the rest.

The method is based on various frequency of presence in DNA of fragments with which binding occurs (bacteria: 1:50; fungi: 1:160; human: 1:2200). Due to enrichment, an essential part of hDNA, virus

DNA as well as a certain part of unbound bacterial and fungal DNA is eliminated; notably the proportion of eliminated DNA strongly depends on bacteria genus (species). The kit is studied in detail in (Glassing 2015). Submucosal part of small intestine biopsies containing a high percentage of human cells (4 samples) is used as sample material. Average hDNA concentration after enrichment fell by 2.4 times, average quantity of 16S copies increased by 3.4 times. Average values of Pearson correlation coefficient, calculated on reads received as a result of 16S-tests before and after LOOXSTER use, are calculated and gathered in Table 17 (read data are borrowed from Annex to Glassing 2015). The chart for sample 4 is given in (fig. 3, Glassing 2015). The authors of the article admit that disruption of bacDNA representation after enrichment is unavoidable.

LOOXSTER method was also applied in other studies, but those works did not carry out qualitative evaluation of enrichment effect on natural samples (a test on artificially created mixture of hDNA and bacDNA was carried out).

One more method of hDNA elimination is based on the use of duplex-specific nuclease DSN (Gijavanekar 2012, Song 2014). Isolated DNA is fragmented, denatured, and then again hybridized in dsDNA. Hybridization primarily takes place for those ssDNA which are found in bigger concentration, which is mainly hDNA. Subsequently DSN is applied, which binds and contributes to degradation of dsDNA only, thereby significantly reducing hDNA representation (for whole blood almost by 100 times) (Song 2014). There have been no researches testing the influence of such elimination on nhDNA representation (the way it is done in Feehery 2013, Glassing 2015 and Marotz 2018). Besides, there is no standard kit based on this method.

5.9. Identifying plastome

Plasma metagenome studies regularly discover plant DNA (henceforth plastDNA). All species of plant DNA in metagenome are generally called plastome. This occurs both while studying WMS-test results and while using unique primers specific to plant genes (Rizzi 2012, Spisak 2013, Levitsky 2016). Normally, the source of plastDNA fragments in blood is DNA from products of plant origin, which is not completely degraded during digestion in small intestine.

Depending on the degree and type of food processing, each person eats up to 1 gram of DNA a day (which is called dietary). A few hours after a meal, most dietary DNA (not less than 95%) is degraded during digestion. Nevertheless, small amounts of dietary DNA, from several hundred to 1700 nucleotides long, do not degrade in GIT and get to various organs through blood and/or lymph flow. Dietary DNA is found in intestine epithelial cells, peripheral leukocytes, cells of spleen and liver. By other estimates, from 10^{-8} to 10^{-7} of all dietary DNA within several hours after a meal are found in plasma and in leukocytic fraction, in fragments of about 300 nucleotides' length on average (Rizzi 2012).

Most papers are devoted to studying DNA income from genetically modified products, both directly from food and through livestock products (Levitsky 2016).

Within the project, it is expected to obtain information on plastome due to a limited (no more than 50) list of genomes of basic plant products being included in the reference catalog (more than 200 in Spisak 2013). The list will be made with due regard to the list of basic plant products, included in the diet recommended to patients for the term preceding sampling of whole blood and biopsies of psoriatic skin (section 5.10. p.2).

Due to this, plastome within the project will be identified and studied as part of metagenome.

All kinds of plastome found in skin samples will be immediately included in NL - non-resident fraction of metagenome MPS (determined logically) (slides *75. nhDNA-MPS* and *76. MPS-example*, Supplement S4.2, section 5.12). This will enable us to conduct complex analysis of whole blood metagenome and metagenome of psoriatic skin (phagocytes) more precisely, and, as it is one of the main objectives of the project, identifying plastomes is justified. Identifying plastomes will not increase the cost and complexity of practical tasks, since it is an analytical task (T3.12 and T5.4).

Information on plastomes of postprandial whole blood and of psoriatic skin (phagocytes) will be obtained for the first time. Information on plastome of whole blood will enable us to check the results obtained for fasting plasma (Spisak 2013).

5.10. Procedure of preparing patients and performing WMS-test of whole blood

1. **Selection of PP and HP.** Only those PP and HP (henceforth patients) who meet the selection criteria can take part (details will be given during the pilot stage of the project). It is recommended to include PP with a broad range of PD severity on PASI, but without infection problems, in the list of participants (Rademaker 2018) in order to minimize income of bacterial products into blood, in addition to those which constantly arrive from small intestine. A wide range of PASI is necessary in order to discover correlations between PASI and nhDNA composition (qualitative and quantitative) of whole blood. Primary selection is

carried out by Organizing Committee of the project, according to the information provided by candidates. This is done by studying Questionnaires, results of examinations (carried out within the last 6 months) and photos intended to estimate PD condition (photos - only for PP).

2. **Preparation for sampling.** Before WMS-test, patients observe SVLD (sterile variant of low-microbial diet) for 5-7 days in order to minimize the presence of transient small intestine microbiome, income of its bacterial products in blood, as well as their accumulation in blood phagocytes. An approximate menu is to be elaborated.

During this period, it is prohibited to take any medicines which can influence GIT microbiome. At least 2 weeks after any infectious disease are to pass before sampling, etc.

3. **Breakfast before the second (postprandial) blood sampling**. For WMS-test and macromolecular small intestine permeability test each patient donates blood twice a day. The first time is on an empty stomach (fasting portion A), the second is a few hours after a substantial breakfast, i.e. postprandially (portion B). The best time for portion B sampling will be specified during the pilot stage. Breakfast menu is to follow SVLD, 1.5 times bigger than normal size, and the food is to be easily digestible. A few versions of this menu with approximately identical caloric value are to be elaborated.

After breakfast, chyme is to pass small intestine up to ileocecal valve and further. The main goal of breakfast is the necessity to nourish the whole small intestine microbiome so as to maximize the rate of bacterial product income (including bacDNA) into blood flow.

Food intake brings about rapid growth of small intestine lumen and parietal microbiome. This, in its turn, causes growth of bacterial product income into blood flow, its peak being notably during microbiome reduction (dying off after growth) in the process of chyme passing to large intestine (Ciampolini 1996, Bala 2014, Erridge 2007, Ghanim 2010, Gnauck 2016a, Milan 2017, Munford 2016).

4. **Sampling.** Each patient donates blood samples twice a day. The first time is on an empty stomach (fasting portion A), the second – in a certain time after breakfast (postprandial portion B). Note that all precautionary measures (Mangul 2016, Païssé 2016, Potgieter 2015) are taken, which make it possible to exclude (minimize) contamination of blood samples by skin microbiome through needle when sampling vein blood, as well as during their storage, transportation and processing. The number and amount of blood samples taken from one patient is dictated by subsequent test requirements. Between the moment of blood sampling and the moment of DNA isolation from it, the blood sample is to be stored and transported in such conditions which minimize degradation rate of bacterial products (both endocyted earlier and contained in biomaterial) by blood phagocytes. For the same purpose, the time between these two moments should be minimized.

Samples of psoriatic skin (several biopsies from each PP) are to be taken right after sampling the postprandial blood portion. Pinpoint plaques are of interest, as they are expected to have high neutrophil concentration and to contain HPV DNA (as one of kebnerization triggers). Such biopsies are preferable on condition of plaque localization on the site with the lowest probability of resident Streptococcus sp. presence (section 2.5).

5. **Samples of NTC**. In order to control contamination level, along with blood samples several samples of NTC (no template control) are formed (section 5.7).

6. **DNA isolation.** Whole blood is studied. All blood cells are destroyed so that all previously endocyted (bound) nhDNA is released from them. When this occurs, hDNA is released as well. Then the sample is cleared of everything, except DNA, in a standard way. One of the standard kits for DNA isolation from blood is going to be used: NucleoSpin Blood (XL,L) (Macherey-Nagel); Blood DNA Isolation (Mini, Midi, Maxi) Kit (Norgen Biotek); innuPREP Blood DNA Master Kit (Analytik Jena AG); Wizard Genomic Purifcation Kit PreAnalytiX (Promega); Blood DNA Kit (PAXgene); QIAamp DNA Blood (Mini, Midi, Maxi) Kit (Qiagen). Which one is going to be used is to be specified during the pilot stage (task T1.3).

Preparation of blood samples plays an essential role. The fastest destruction of blood cells after its sampling terminates degradation processes of earlier endocyted nhDNA, which ensures maximizing total bacDNA representation. In (Li 2018) a special buffer was used, which ensured maximum bacDNA representation (section 2.2). Minimal contamination level of reagents (task T1.6) and DNA fragmentation parameters (median and deviation of distribution) will be important criteria of choosing the kit. For more detail see section 5.7.

7. **Determining concentration of all DNA and bacDNA (blood and NTC).** Concentration of all DNA (in postprandial portion B) and bacDNA (in fasting portion A, in postprandial portion B, and in sample of NTC) is determined. This is necessary to prepare biomaterial for sequencing, to normalize WMS-test results, to control contamination level, and to determine macromolecular small intestine permeability.

Concentration of all DNA is carried out on Qubit Fluorometric Quantitation, bacDNA concentration – with the use of Femto™ Bacterial DNA Quantification Kit (E2006) (a uniform method for blood samples and samples of NTC). Fasting blood (portion A) is not used afterwards.

Determining concentration of all DNA and bacDNA in blood samples and NTC in the course of their preparation for library formation and sequencing is carried out several times. One of main goals of it is controlling contamination level (section 3.2.1.)

The method of "internal standard" can be alternatively applied: - adding DNA of characteristic bacterium to DNA blood sample (e.g. 1% of all DNA amount). It is such a bacterium whose DNA definitely cannot be present in this biomaterial (Tan 2015). This will make it possible to normalize correlation between its concentration in the sample and the quantity of reads to be mapped on its genome according to WMS-test.

8. **Determining macromolecular small intestine permeability.** The relation of CB - bacDNA concentration in postprandial blood to CA - concentration in fasting blood is a value characterizing macromolecular small intestine permeability (8). Task T2.3 consists in determining this dimensionless value for each patient. Its possibility is going to be explored during the pilot stage (task T1.5). Such an approach is similar to determining small intestine permeability by ovalbumin test (Parfenov 1999, Mazo 2008, Peslyak 2012a).

(8) Qsimp = CB / CA

Qsimp can be used to calculate NORM coefficient (9) for the purpose of approximating Qminus, Qlpsm, Qplus, Qpsb, Qlpsb, Qpgb parameters (task of T3.9) (determined for postprandial blood) to the daily average ones. Such normalizing is necessary if we assume that PD severity on PASI better correlates with the average daily values of these variables. Within the project it is not expected to carry out daily monitoring of bacDNA concentration (for this purpose the patients would have to stay in hospital and to donate blood repeatedly).

(9) NORM = (1 + Qsimp)/(2*Qsimp)

9. **hDNA elimination.** Postprandial blood. hDNA is maximally eliminated from the sample, but in such a way that nhDNA is retained. The purpose is maximum nhDNA enrichment (concentration increasing) in the sample (with maximum preservation of minor species representation). Labor intensity, quality and degree of result reliability of subsequent WMS-test (Song 2014) depend on how successful and thorough this enrichment is.

There are many various methods of preliminary hDNA elimination (Applications 2015, Archer 2010, Ferretti 2017, Fitting 2012, Gyarmati 2016, Opota 2015, Song 2014, Yigit 2016, Zhou 2012). For the project objectives, only two standard kits are suitable: NebNext Microbiome DNA Enrichment and LOOXSTER® Enrichment Kit (section 5.7, task T1.4.1).

10. **WMS-test.** For the sample obtained after hDNA elimination, standard WMS-test is carried out. The obtained results are processed: reads of poor quality, reads belonging to hDNA, etc. are removed. Subsequently assembling the remaining reads in contigs and mapping on pre-arranged reference is carried out.

As a result, we will get representation of each nhDNA. nhDNA concentration in whole blood is calculated after normalization, proceeding from what method has been chosen (see point 6 above).

WMS-tests carried out for samples of NTC will enable us to estimate contamination level and it's composition and to exclude from metagenomes information on those nhDNA whose representation is comparable to contamination level and/or which are most likely to be contaminants.

11. **Determining PAMP-nemia level.** (Table 8. Tasks T2.4 and T2.5).

12. **bacDNA and plastDNA – analytics and search of correlations.** (Table 8. Tasks T3).

13. **nhDNA (non-bacDNA).** WMS-test enables us to elicit DNA of fungi, helminths, viruses, phages. To do this, it will be necessary to perform mapping for an additional reference. If a considerable amount of DNA of fungi, helminthes and viruses is discovered, additional research intended to search for localization of infectious process is to be made.

5.11. Isolation of DNA from psoriatic skin (phagocytes)

In the course of preparation of this project version, it became clear that procedure of isolation of phagocytes from psoriatic skin (slide *77. Phagocytes selection*) has certain disadvantages. This is a preparatory procedure for all DNA isolation from skin phagocytes. Notably, the ultimate aim is detecting

nhDNA of non-resident origin (i.e. that which came to skin in blood phagocytes) with the greatest possible concentration.

In case of isolating skin phagocytes from cellular suspension in an immunomagnetic or other way, quite a lot of time passes before it is possible to start destruction of phagocytes and DNA isolation (Ferretti 2017, Garcia-Garcera 2013, Meisel 2016, Oh 2014).

To produce cellular suspension, Whole-skin-dissociation-kit-human (Miltenyi Biotec) can be applied. The minimum time to produce cellular suspension is 3 hours. Subsequently, phagocytes (e.g. neutrophils) are to be isolated from skin suspension. EasySep™ Human CD15 Positive Selection Kit (STEMCELL Technologies) is appropriate for the purpose (for positive selection). Its performance takes at least an hour. In case of isolating all types of phagocytes (neutrophils, monocytes, macrophages and dendritic cells), cocktail for positive immunomagnetic selection containing antibodies to CD11c, CD14, CD15 and CD16 can be applied.

As a result, not less than 4 hours pass until we can start destroying phagocytes and isolating DNA contained in them. During this period, live phagocytes are kept at 37C° and continue degradation of everything they endocyted earlier. And they can degrade a very high percentage of non-resident nhDNA which was in them at the moment of biopsy sampling …

Phagocytes of psoriatic skin (in biopsies of 1 mm deep) at moderate-severe psoriasis make up to 10-15% of the total number of cells. It is clear that by isolating phagocytes from other non-phagocytic cells of skin, we facilitate the work for subsequent NME use, as hDNA amount in samples becomes 7-10 times smaller (since non-phagocytic cells are discarded). But what if during this procedure (obtaining skin suspension and immunomagnetic isolation of phagocytes from it) the amount of required nhDNA falls (due to its degradation in live phagocytes) by a larger number of times?

An alternative to DNA isolation from previously isolated phagocytes is immediate DNA isolation from biopsy. In this case, however, apart from "excess" hDNA we will also get "excess" nhDNA of resident origin. There is more than enough of such nhDNA in skin, including that outside cells and even in derma (*39. Derm-16S-1* and *40. Derm-16S-2*). This is normal skin in which there are hardly any phagocytes of non-resident origin, so all bacDNA is from bacteria of resident origin (notably those outside CD11c+DC) (Nakatsuji 2013).

The optimum protocol will be formulated after solving tasks T1.4.1 and (if situation requires) T1.7.

Let us enumerate the advantages of identifying metagenome of psoriatic skin instead of metagenome of psoriatic skin phagocytes.

Firstly, YN-model presupposes finding nhDNA of non-resident origin in intercellular space as a result of netosis. When identifying metagenome of psoriatic skin phagocytes, this part will be lost.

Secondly, when identifying metagenome of psoriatic skin there will remain a possibility of identifying virome of all skin (particularly HPV), since keratinocytes will not be excluded. When identifying metagenome of psoriatic skin phagocytes, an essential part of virome will be lost (particularly HPV, which mainly infects keratinocytes).

Thirdly, if task T1.4.1 is successfully solved (fractional bacDNA enrichment from whole blood DNA), it is also likely to be effective for bacDNA enrichment from psoriatic skin biopsy DNA. Specifically, this is so because it will enable us to exclude high-molecular fractions not only of hDNA, but also of bacDNA of resident origin.

5.12. Supplements

A. Metagenomes of blood and psoriatic skin. Research project. Presentation and illustrations. (e2.2, pdf-format, DOI: 10.5281/zenodo.2668376)

B. Metagenomes of blood and psoriatic skin. Research project. Supplements S1-S8. (e2.2, pdf-format, DOI: 10.5281/zenodo.2668459)

S1. Comparative characteristics of 16S and WMS-tests
S2. Determining nhDNA concentration by WMS-test results
S3. Alternative scheme of blood sampling
S4. Algorithm of MPS division into fractions
S5. Statement and analysis of math task about nhDNA in two unidirectionally connected tanks
S6. Y-model and YN-model. Hypotheses.
S7. Resources of metagenomic research and sequencing
S8. Notation and definition for variables, arrays and sets

C. Video “Immunology in the skin”

6. List of illustration (slides)

All illustrations are in Supplement A (section 5.12). The main symbols used in illustrations are collected on slide *11. Symbols*.

Number and short name	Full name
1. Section1	**Section 1. SIBO (Small intestine bacterial overgrowth) at psoriatic disease. Presumed Y-antigen and peptides. PsB - bacteria presumed psoriagenic. Systemic models of psoriasis pathogenesis (BF-model, Y-model and YN-model). Systemic psoriatic process SPPN and checked hypotheses.**
2. Psoriatic_disease	Psoriatic and normal skin
3. Patient_Stat-C	Statistics of PD incidence on countries
4. Patient_Stat-R	Incidence statistics in Russian Federation. Estimated number of psoriatic patients (PP) in world.
5. Basic_research	Basic researches
6. Permeability-1	Transcellular small intestine permeability at psoriasis. D-xylose test.
7. Permeability-2	Intercellular small intestine permeability at psoriasis. Ovalbumin test.
8. SIBO-1	SIBO (small intestinal bacterial overgrowth) at psoriasis
9. SIBO-2	SIBO. Transient microflora of proximal small intestine.
10. SIBO_Moscow	Small intestine microbiome of PP in Treitz ligament, lg (CFU/ml).
11. Symbols	Symbols
12. BF-model	BF-model of pathogenesis (B.Baker & L.Fry, 2006-7).
13. Antigen-1	Mature dendritic cell present unknown Y-antigen to T-lymphocyte
14. Antigen-2	Versions of origin of unknown antigen
15. PAMP,TLR,NOD	PAMP, structure and localization of TLR2, TLR4, TLR9, NOD1 and NOD2
16. PG_PsB-1	Peptidoglycan (PG) structure and PsB
17. PG_PsB-2	Biosynthesis of peptidoglycan
18. PG_PsB-3	Species of Gram+ bacteria with interpeptide bridges IB-Y. IB-Y = (L-Ala)-(L-Ala) or (L-Ser)-(L-Ala). (KEGG database).
19. PG_PsB-4	Muropeptides and peptides which are formed at degradation of Str.pneumonia peptidoglycan
20. PG_PsB-5	B13-2 peptide - potential Y-antigen
21. PG_PsB-6	Y-B11 peptide - potential Y-antigen
22. SPPN-PAMP-nemia	YN-model. Systemic psoriatic process SPPN and PAMP-nemia.
23. SPPN	YN-model. Systemic psoriatic process SPPN. Interaction of subprocesses.
24. Local_processes_Y	Y-model. Attraction from blood and transformation of monocytes and dendritic cells in psoriatic derma.
25. Local_processes_YN	YN-model. Attraction of neutrophils from blood and netosis some of them. Endocytosis and presentation Y-antigens lost by Neu-Y during netosis.
26. YN-model	YN-model of pathogenesis. Interaction of local processes.
27. NCS1_Hypo	4 hypotheses (H1-1, H2, HN3 and HN10) on check
28. Biotransfer	Presence and movement of non-host biomaterial between organs.
29. HN10-S	Non-host biomaterial comes to psoriatic skin inside blood phagocytes (hypothesis HN10-S)
30. Section2	Section 2. Metagenomic sequencing. Blood metagenome. Skin metagenome.

31. Okubo	Total bacDNA in blood monocytes of PP and HP (16S-test, Okubo 2002)
32. Blood_Psor	BacDNA in blood plasma of psoriatic patients (16S-test, Munz 2010)
33. Blood_WMS_and_Culture	Pathogens identified by cultural method (BC) and NGS (WMS-test) in blood plasma (Long 2016)
34. Blood-bacDNA (France)	BacDNA in whole blood (30 HP, 16S-test, Paisse 2016)
35. Blood-Germany-1	Characteristics of blood plasma (7 patients with sepsis, 12 HP, WMS-test, Grumaz 2016)
36. Blood-Germany-2	Blood plasma metagenome (12 HP, WMS-test, Grumaz 2016)
37. Blood-bacDNA (China)	BacDNA in whole blood (12 HP, 16S-test, Li 2018)
38. Skin-bacDNA	Bacterial DNA in psoriatic and healthy skin (10 PP, 12 HP, 16S-test, Fahlen 2012)
39. Derm-16S-1	Bacterial DNA in epidermis and derma of non-psoriatic patients (16S-test, Nakatsuji 2013) - 1
40. Derm-16S-2	Bacterial DNA in epidermis and derma of non-psoriatic patients (16S-test, Nakatsuji 2013) -2
41. Healthy-skin	Microorganisms (including bacteria and bacDNA) in healthy skin. Assumptions and facts.
42. Skin_Bacteria_3D	Bacterial DNA on healthy skin men (1) and women (2). (16S-test, Bouslimani 2015).
43. Skin-WMS-18-1	Skin biogeography (15 HP, WMS-test, Oh 2014). Smears from 18 sites.
44. Skin-WMS-18-2	Skin biogeography (15 HP, WMS-test, Oh 2014). Main results.
45. Skin-WMS-18-3	Skin biogeography (15 HP, WMS-test, Oh 2014). Specification for two widespread species (P.acnes and Staph.epidermidis) to within strains.
46. Skin-AtD-WMS-genus	Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of several genera.
47. Skin-AtD-WMS-Strep	Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Streptococcus sp.
48. Skin-AtD-WMS-Staph	Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Staphylococcus sp.
49. Skin-AtD-WGS-Strain	Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Staphylococcus aureus strains.
50. Psorskin-swab-WMS-1	Skin metagenome (28 PP, smears, WMS-test, Tett 2017). Representation of species.
51. Psorskin-swab-WMS-2	Skin metagenome (28 PP, smears, WMS-test, Tett 2017). Similarity and difference of strains composition.
52. Psorskin-6	Skin metagenome (114 PP, smears, 16S-test, Loesche 2018). Composition and representation of main taxons.
53. Section3	**Section 3. Phagocytes of normal and psoriatic skin. NET - neutrophil extracellular traps in blood and in psoriatic skin. New models of psoriasis pathogenesis.**
54. Skin_2D	Dendritic cells, macrophages and T-lymphocytes in healthy skin
55. Skin_3D	Dendritic cells, macrophages and T-lymphocytes in healthy skin (3D)
56. Phagocytes-1	Attraction of blood phagocytes in skin at moderate-severe psoriasis
57. Macrophages	Macrophages in healthy and psoriatic skin
58. Dendritic_Cells	Dendritic cells in healthy and psoriatic skin
59. Neutrophils	Neutrophils in healthy and psoriatic skin
60. Neutrophils-Munro	Neutrophils in psoriatic epidermis (Munro's abscesses)
61. Netosis	Netosis - formation of NET (neutrophil extracellular traps)
62. Net-blood-1	NET in healthy and psoriatic blood (Lin 2011)
63. Net-blood-2	NET in healthy and psoriatic blood (Hu 2016)

64. Net-blood-3	Correlation between PASI and percentage of netotic blood neutrophils (Hu 2016)
65. Net-skin-1	NET in psoriatic skin (Lin 2011)
66. Net-skin-2	NET in psoriatic epidermis (Hu 2016)
67. KB-schema	First scheme of psoriasis pathogenesis taking into account NET (Lin 2011)
68. FM-model	Model of psoriasis pathogenesis taking into account NET and MSET (Delgado-Rizo 2017)
69. SE-model	Model of psoriasis pathogenesis taking into account NET (Schon 2018)
70. GKH-model	Model of psoriasis pathogenesis without NET (Hawkes 2017)
71. BMM-model	Model of psoriasis pathogenesis without Neu and NET (Benhadou 2018)
72. Section4	**Section 4. Complex study of metagenomes of blood and psoriatic skin. Methods and problems of host DNA elimination.**
73. 2Pools-D	Whole blood metagenome and metagenome of psoriatic skin (phagocytes) in dynamics
74. 2Pools-S	Whole blood metagenome and metagenome of psoriatic skin (phagocytes) at its stable state. Instant cut.
75. nhDNA-MPS	Presumed fractions of MPS – metagenome of psoriatic skin (phagocytes)
76. MPS-example	MPS division algorithm - metagenome of psoriatic skin (phagocytes) into fractions and subsets. Example.
77. Phagocytes selection	Selection of phagocytes from psoriatic skin by immunomagnetic method
78. NME-1	Preparation and subsequent enrichment of blood samples by NebNext Microbiome Enrichment kit
79. NME-1a	Integrity (fragment distribution) of DNA isolated from whole blood
80. NME-2	Non-host DNA selection from biomaterial with predominant host DNA content (blood or skin cells). NebNext Microbiome Enrichment kit.
81. NME-3	Enrichment of saliva and blood samples by NebNext Microbiome Enrichment kit
82. NME-4	hDNA elimination from saliva samples in several ways, including NME (HP, n=8, Marotz 2018)
83. Section5	**Section 5. Order of patients' participation. Main questions and novelty.**
84. Part_Order_NCS1	Order of participation of psoriatic patients (PP) and healthy persons (HP) in NCS1 project.
85. Stage1-Q1	Question 1. Does severity of psoriatic disease correlate with concentration of any nhDNA in whole blood and/or with PAMP-nemia level?
86. Stage1-Q2	Question 2. Does non-degraded nhDNA come from blood into psoriatic skin?
87. Stage1-Q1&2	Project NCS1. Two main questions.
88. Stage1-new	Project NCS1. What novelty consists in?

7. Bibliography

Adib-Conquy M, Cavaillon JM. Compensatory anti-inflammatory response syndrome. Thromb Haemost. 2009 Jan;101(1):36-47. 19132187.

Albanesi C, Scarponi C, Bosisio D, Sozzani S, Girolomoni G. Immune functions and recruitment of plasmacytoid dendritic cells in psoriasis. Autoimmunity. 2010 Apr;43(3):215-9. 20166874.

Albanesi C, Scarponi C, Pallotta S. et al. Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. J Exp Med. 2009 Jan 16;206(1):249-58. 19114666.

Alekseyenko AV, Perez-Perez GI, De Souza A. et al. Community differentiation of the cutaneous microbiota in psoriasis. Microbiome. 2013 Dec 23;1(1):31. 24451201.

Alyami H. NOD1/NOD2 Upregulation in Fusobacterium Nucleatum Induced NETosis. (2018). Dental Teses. 29, link.

Amar J. Lange C. Payros G. et al. Blood microbiota dysbiosis is associated with the onset of cardiovascular events in a large general population: the D.E.S.I.R. study. PLoS One. 2013;8(1):e54461. 23372728.

Amar J, Serino M, Lange C. et al. Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. Diabetologia. 2011 Dec;54(12):3055-61. 21976140.

Applications of Clinical Microbial Next-Generation Sequencing. Report on an American Academy of Microbiology Colloquium held in Washington, DC, in April 2015. p.56. link.

Arakawa A, Siewert K, Stohr J. et al. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med. 2015 Dec 14;212(13):2203-12. 26621454.

Aransay AM, Trueba JLL. Field Guidelines for Genetic Experimental Designs in High-Throughput Sequencing. Springer International Publishing Switzerland, 2016, 399 p. ISBN: 978-3-319-31350-4.

Archer MJ, Long N, Lin B. Effect of probe characteristics on the subtractive hybridization efficiency of human genomic DNA.BMC Res Notes. 2010 Apr 20;3:109 20406484.

Aucamp J, Bronkhorst AJ, Badenhorst CPS, Pretorius PJ. The diverse origins of circulating cell-free DNA in the human body: a critical re-evaluation of the literature. Biol Rev Camb Philos Soc. 2018 Aug;93(3):1649-1683. 29654714.

a Baker BS, Laman JD, Powles AV. et al, Peptidoglycan and peptidoglycan-specific Th1 cells in psoriatic skin lesions, J Pathol 2006 Jun;209(2):174-81. 16493599.

b Baker BS, Powles A, Fry L. Peptidoglycan: a major aetiological factor for psoriasis? Trends Immunol. 2006 Dec;27(12):545-51. 17045843.

c Baker BS. Skin Immune Mechanisms in Health and Disease, Garner Press, 2006, 328 p. ISBN 0955160308.

Baker BS. From Arsenic To Biologicals: A 200 Year History Of Psoriasis, Garner Press, 2008, 128 p. ISBN 978-0955160325.

Bala S, Marcos M, Gattu A. et al. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. PLoS One. 2014 May 14;9(5):e96864. 24828436.

Barreto-Bergter E, Figueiredo RT. Fungal glycans and the innate immune recognition. Front Cell Infect Microbiol. 2014 Oct 14;4:145. 25353009.

Benhadou F, Mintoff D, del Marmol V Psoriasis: Keratinocytes or Immune Cells – Which Is the Trigger? Dermatology 2018. doi: 10.1159/000495291.

Bhat S, Emslie KR. Digital polymerase chain reaction for characterisation of DNA reference materials. Biomol Detect Quantif. 2016 May 3;10:47-49. 27990349.

Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol. 2009 Oct;30(10):475-87. 19781994.

Blanchet X, Langer M, Weber C, Koenen RR, von Hundelshausen P. Touch of chemokines. Front Immunol. 2012 Jul 12;3:175. 22807925.

Bogunovic M, Ginhoux F, Wagers A et al. Identification of a radio-resistant and cycling dermal dendritic cell population in mice and men. J Exp Med. 2006 Nov 27;203(12):2627-38. 17116734.

Bouslimani A, Porto C, Rath CM et al. Molecular cartography of the human skin surface in 3D. Proc Natl Acad Sci U S A. 2015 Apr 28;112(17):E2120-9. 25825778.

Bryzgunova OE, Laktionov PP, Generation of blood circulating DNA: the sources, peculiarities of circulation and structure. Biomedical Chemistry, ISSN: 2310-6972, 2015;(4): 409-426. (Rus). elib.

Bui NK, Eberhardt A, Vollmer D. et al. Isolation and analysis of cell wall components from Streptococcus pneumoniae.Anal Biochem. 2012 Feb 15;421(2):657-66. 22192687.

Cheung KL, Jarrett R, Subramaniam S. et al. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med. 2016 Oct 17;213(11):2399-2412. 27670592.

Chng KR, Tay AS, Li C. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat Microbiol. 2016 Jul 11;1(9):16106. 27562258.

Ciampolini M, Bini S, Orsi A. Microflora persistence on duodenum-jejunal flat or normal mucosa in time after a meal in children. Physiol Behav. 1996 Dec;60(6):1551-6. 8946504.

Clark SR, Ma AC, Tavener SA. et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med. 2007 Apr;13(4):463-9. 17384648.

Codoñer FM, Ramírez-Bosca A, Climent E, et al. Gut microbial composition in patients with psoriasis. Sci Rep. 2018 Feb 28;8(1):3812. 29491401.

Christophers E, Metzler G, Röcken M. Bimodal immune activation in psoriasis. Br J Dermatol. 2014 Jan;170(1):59-65. 24117368.

Dalpke A1, Frank J, Peter M, Heeg K. Activation of Toll-Like Receptor 9 by DNA from different bacterial species. Infect Immun. 2006 Feb;74(2):940-6. 16428738.

Davey MS, Morgan MP, Liuzzi AR. et al. Microbe-specific unconventional T cells induce human neutrophil differentiation into antigen cross-presenting cells. J Immunol. 2014 Oct 1;193(7):3704-16. 25165152.

De Jongh GJ, Zeeuwen PL, Kucharekova M. et al., High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis J Invest Dermatol. 2005 Dec;125(6):1163-73. 16354186.

De Yang, Chen Q, Schmidt AP. et al. LL37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med. 2000 Oct 2;192(7):1069-74. 11015447.

Delgado-Rizo V, Martínez-Guzmán MA, Iñiguez-Gutierrez L. et al. Neutrophil Extracellular Traps and Its Implications in Inflammation. Front Immunol. 2017 Feb 6;8:81. 28220120.

Dinakaran V, Rathinavel A, Pushpanathan M. et al. Elevated levels of circulating DNA in cardiovascular disease patients: metagenomic profiling of microbiome in the circulation. PLoS One. 2014 Aug 18;9(8):e105221. 25133738.

Duan H, Koga T, Kohda F, Hara H, Urabe K, Furue M. Interleukin-8-positive neutrophils in psoriasis., J Dermatol Sci. 2001 Jun;26(2):119-24. 11378328.

Ely PH. Is psoriasis a bowel disease? Successful treatment with bile acids and bioflavonoids suggest it is. Clin Dermatol. 2018 May - Jun;36(3):376-389. 29908580.

Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr. 2007 Nov;86(5):1286-92. 17991637.

Fahlen A, Engstrand L, Baker BS, Powles A, Fry L. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. Arch Dermatol Res. 2012 Jan;304(1):15-22. 22065152.

Federal clinical guidelines for management of patients with psoriasis. RODVK, Moscow. 2015, 59 p., (rus), link, FEML

Feehery GR, Yigit E, Oyola SO. et al. A method for selectively enriching microbial DNA from contaminating vertebrate host DNA. PLoS One. 2013 Oct 28;8(10):e76096. 24204593.

Ferretti P, Farina S, Cristofolini M. et al. Experimental metagenomics and ribosomal profiling of the human skin microbiome. Exp Dermatol. 2017 Mar; 26(3):211-219. 27623553.

Filipe SR, Severina E, Tomasz A. Distribution of the mosaic structured murM genes among natural populations of Streptococcus pneumoniae. J Bacteriol. 2000 Dec;182(23):6798-805. 11073926.

Filipe SR, Severina E, Tomasz A. Functional analysis of Streptococcus pneumoniae MurM reveals the region responsible for its specificity in the synthesis of branched cell wall peptides. J Biol Chem. 2001 Oct 26;276(43):39618-28. 11522792.

Fitting C, Parlato M, Adib-Conquy M. et al. DNAemia detection by multiplex PCR and biomarkers for infection in systemic inflammatory response syndrome patients. PLoS One. 2012;7(6):e38916. 22719987.

Fiser A, Filipe SR, Tomasz A. Cell wall branches, penicillin resistance and the secrets of the MurM protein. Trends Microbiol. 2003 Dec;11(12):547-53. 14659686.

Fomina EC. Association of HPV and staphilococci in the formation of disturbances of microflora of skin at psoriasis. Dissertation, Moscow, 2009, 110 p., (Rus), link.

Frey KG, Bishop-Lilly KA. Next-Generation Sequencing for Pathogen Detection and Identification. Chapter 15 in Methods in Microbiology. Volume 42, 2015, 525–554. ISSN 0580-9517. link.

Fry L. Microbiome of chronic plague psoriasis. Chapter 21 in The Human Microbiota and Chronic Disease: Dysbiosis as a Cause of Human Pathology. 2016, John Wiley & Sons, Inc. ISBN 9781118982877, link.

Fry L, Baker BS, Powles AV, Fahlen A, Engstrand L. Is chronic plaque psoriasis triggered by microbiota in the skin? Br J Dermatol. 2013 Jul;169(1):47-52. 23521130.

Fuentes-Duculan J, Suárez-Fariñas M, Zaba LC et al. A Subpopulation of CD163-Positive Macrophages Is Classically Activated in Psoriasis. Journal of Investigative Dermatology 2010 Oct; 130:2412-2422. 20555352.

Fuentes-Duculan J, Bonifacio KM, Hawkes JE. et al. Autoantigens ADAMTSL5 and LL37 are significantly upregulated in active Psoriasis and localized with keratinocytes, dendritic cells and other leukocytes. Exp Dermatol. 2017 Nov;26(11):1075-1082. 28482118.

Fukui H. Endotoxin and Other Microbial Translocation Markers in the Blood: A Clue to Understand Leaky Gut Syndrome. Cell Mol Med 2016, 2:3. link.

Gambichler T, Skrygan M, Tomi NS. et al. Differential mRNA Expression of Antimicrobial Peptides and Proteins in Atopic Dermatitis as Compared to Psoriasis Vulgaris and Healthy Skin. Int Arch Allergy Immunol. 2008 Apr 30;147(1):17-24. 18446049.

Ganguly D, Chamilos G, Lande R et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med. 2009 Aug 31;206(9):1983-94. 19703986.

Gao Z, Tseng CH, Strober BE. et al. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. PLoS ONE. 2008 Jul 23;3(7):e2719. 18648509.

Garaeva ZS, Safina NA, Tiurin IA, Kuklin VT, Zinkevich OD. Intesitnal dysbiosis as the cause of systemic endotoxemia in psoriatic patients. Vestn Dermatol Venerol, 2007;(1): 23-27. (Rus), ISSN 0042-4609, elib.

Garcia-Garcera M, Garcia-Etxebarria K, Coscolla M, Latorre A, Calafell F. A new method for extracting skin microbes allows metagenomic analysis of whole-deep skin. PLoS One. 2013 Sep 20;8(9):e74914. 24073227.

Gaspari AA, Tyring SK, Kaplan DH, Clinical and Basic Immunodermatology. Springer, 2017, 889 p. ISBN 978-3-319-29785-9.

Gijavanekar C, Strych U, Fofanov Y, Fox GE, Willson RC. Rare target enrichment for ultrasensitive PCR detection using cot-rehybridization and duplex-specific nuclease. Anal Biochem. 2012 Feb 1;421(1):81-5. 22155054.

Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. Curr Opin Immunol. 2008 Aug;20(4):401-7. 18611439.

Gillitzer R, Wolff K, Tong D et al. MCP-1 mRNA expression in basal keratinocytes of psoriatic lesions. J Invest Dermatol. 1993 Aug;101(2):127-31. 8345212.

Ginhoux F, Collin MP, Bogunovic M et al. Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state. J Exp Med. 2007 Dec 24;204(13):3133-46. 18086862.

Ghanim H, Sia CL, Upadhyay M. et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. Am J Clin Nutr. 2010 Apr;91(4):940-9. 20200256.

Glassing A, Dowd SE, Galandiuk S. et al. Changes in 16s RNA Gene Microbial Community Profiling by Concentration of Prokaryotic DNA.J Microbiol Methods. 2015 Dec;119:239-42. 26569458.

Glassing A, Dowd SE, Galandiuk S, Davis B, Chiodini RJ. Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. Gut Pathog. 2016 May 26;8:24. 27239228.

a Gnauck A, Lentle RG, Kruger MC. Chasing a ghost?--Issues with the determination of circulating levels of endotoxin in human blood. Crit Rev Clin Lab Sci. 2016;53(3):197-215. 26732012.

b Gnauck A, Lentle RG, Kruger MC. The Characteristics and Function of Bacterial Lipopolysaccharides and Their Endotoxic Potential in Humans. Int Rev Immunol. 2016 May 3;35(3):189-218. 26606737.

Gosiewski T, Ludwig-Galezowska AH, Huminska K. et al. Comprehensive detection and identification of bacterial DNA in the blood of patients with sepsis and healthy volunteers using next-generation sequencing method - the observation of DNAemia. Eur J Clin Microbiol Infect Dis. 2017 Feb;36(2):329-336. 27771780.

Gosiewski T, Szała L, Pietrzyk A. et al. Comparison of methods for isolation of bacterial and fungal DNA from human blood. Curr Microbiol. 2014 Feb;68(2):149-55. 24026449.

Gottlieb AB, Chamian F, Masud S. et al. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. J Immunol. 2005 Aug 15;175(4):2721-9. 16081850.

Grayson PC, Kaplan MJ. At the Bench: Neutrophil extracellular traps (NETs) highlight novel aspects of innate immune system involvement in autoimmune diseases. J Leukoc Biol. 2016 Feb;99(2):253-64. 26432901.

Greenlee-Wacker MC. Clearance of apoptotic neutrophils and resolution of inflammation. Immunol Rev. 2016 Sep;273(1):357-70. 27558346.

Grice EA, Kong HH, Renaud G. et al. A diversity profile of the human skin microbiota. Genome Res. 2008 Jul;18(7):1043-50. 18502944.

Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011 Apr;9(4):244-53. 21407241.

Grumaz S, Stevens P, Grumaz C. et al. Next-generation sequencing diagnostics of bacteremia in septic patients. Genome Med. 2016 Jul 1;8(1):73. 27368373.

a Gumayunova NG. Syndrome of small intestine bacterial overgrowth at psoriatic disease against blastocystic invasion. Dissertation, Chelyabinsk, 2009, 169 p, (Rus), link.

b Gumayunova NG, Potaturkina-Nesterova NI, Nesterov AS, Magomedov MA. New approaches to diagnosis intestinal dysbiosis of patients who have psoriatic disease. Bulletin of Russian Peoples Friendship University. Medicine. 2009;(2):93-97. (Rus). elib.

c Gumayunova NG. Revealing of small intestine bacterial overgrowth at psoriatic disease. The postgraduate bulletin of the Volga region, 2009 (3-4):162-4. (Rus). elib.

Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis-Part II: Immune cell subsets and therapeutic concepts. J Allergy Clin Immunol., 2011 Jun;127(6):1420-32. 21419481.

Gyarmati P, Kjellander C, Aust C. et al. Metagenomic analysis of bloodstream infections in patients with acute leukemia and therapy-induced neutropenia. Sci Rep. 2016 Mar 21;6:23532. 26996149.

Hannigan GD, Meisel JS, Tyldsley AS. et al. The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. MBio. 2015 Oct 20;6(5):e01578-15. 26489866.

Harkov EI, Prohorenkov VI, Shiryaeva YuA. Indices of functional activity of small intestine in patients with psoriasis. Siberian Medical Review, 2008;(6):55-58. (Rus). elib.

Harkov EI, Shiryaeva YuA, Teryoshina DS. Malabsoption syndrome and psoriasis: the method of correction. Siberian Medical Journal (Irkutsk), 2006;(7):61-63. (Rus). elib.

Harkov EI, Shiryaeva YuA. Malabsorption syndrome in psoriasis: clinical-laboratory parallels. Siberian Medical Review, 2005;(2-3):62-64. (Rus). elib.

Hasler P, Giaglis S, Hahn S. Neutrophil extracellular traps in health and disease. Swiss Med Wkly. 2016 Oct 10;146:w14352. 27723901.

Hawkes JE, Chan TC, Krueger JG. Psoriasis pathogenesis and the development of novel targeted immune therapies. J Allergy Clin Immunol. 2017 Sep;140(3):645-653. 28887948.

Herster F, Bittner Z, Dickhoefer S. et al, RNA-antimicrobial peptide complexes fuel a TLR- and NETosis mediated inflammatory cycle of neutrophil activation in psoriasis. bioRxiv 324814; Epub Sep 5, 2018, doi: https://doi.org/10.1101/324814.

Holler E, Butzhammer P, Schmid K. et al. Metagenomic Analysis of the Stool Microbiome in Patients Receiving Allogeneic Stem Cell Transplantation: Loss of Diversity Is Associated with Use of Systemic Antibiotics and More Pronounced in Gastrointestinal Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2014 May;20(5):640-5. 24492144.

Horiba K, Kawada JI, Okuno Y. et al. Comprehensive detection of pathogens in immunocompromised children with bloodstream infections by next-generation sequencing. Sci Rep. 2018 Feb 28;8(1):3784. 29491477.

Hu SC, Yu HS, Yen FL. et al. Neutrophil extracellular trap formation is increased in psoriasis and induces human β-defensin-2 production in epidermal keratinocytes. Sci Rep. 2016 Aug 5;6:31119, 27493143.

Huh WK, Oono T, Shirafuji Y. et al. Dynamic alteration of human beta-defensin 2 localization from cytoplasm to intercellular space in psoriatic skin. J Mol Med. 2002 Oct;80(10):678-84. 12395153.

Ishihata K, Kakihana Y,Yasuda T.et al. Newly Developed Endotoxin Measurement Method (the Endotoxin Activity Assay) May Reflect the Severity of Sepsis. Open Journal of Pathology, Vol. 3 No. 1, 2013, pp. 1-6. link

Jagielski T, Rup E, Ziółkowska A. et al. Distribution of Malassezia species on the skin of patients with atopic dermatitis, psoriasis, and healthy volunteers assessed by conventional and molecular identification methods. BMC Dermatol. 2014 Mar 7;14(1):3. 24602368.

James C, Iwasiow RM, Birnboim HC. Human genomic DNA content of saliva samples collected with the Oragene self-collection kit. PD-WP-011 Issue 4/2011-11, link.

Jansen PA, Rodijk-Olthuis D, Hollox EJ. et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS ONE. 2009;4(3):e4725. 19266104.

Jozsef L, Khreiss T, Filep JG. CpG motifs in bacterial DNA delay apoptosis of neutrophil granulocytes. FASEB J. 2004 Nov;18(14):1776-8. 15345690.

Jovel J, Patterson J, Wang W. et al. Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. Front Microbiol. 2016 Apr 20;7:459. 27148170.

Kabashima K. Immunology of the Skin. Basic and Clinical Sciences in Skin Immune Responses. 2016, Springer Japan, 510 p. ISBN 978-4-431-55855-2.

Karaji N, Sattentau QJ. Efferocytosis of Pathogen-Infected Cells. Front Immunol. 2017 Dec 22;8:1863. 29312342.

Karlsson FH, Fåk F, Nookaew I, Tremaroli V, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun. 2012;3:1245. 23212374.

Kenny EF, Herzig A, Krüger R. et al. Diverse Stimuli engage different Neutrophil Extracellular Trap pathways. Elife. 2017 Jun 2;6. pii: e24437. 28574339.

Kimer N, Pedersen JS, Tavenier J. et al. Rifaximin has minor effects on bacterial composition, inflammation, and bacterial translocation in cirrhosis: A randomized trial. J Gastroenterol Hepatol. 2018 Jan;33(1):307-314. 28671712.

Kisiel B, Kisiel K, Szymański K. et al. The association between 38 previously reported polymorphisms and psoriasis in a Polish population: High predicative accuracy of a genetic risk score combining 16 loci. PLoS One. 2017 Jun 15;12(6):e0179348. 28617847.

Kim D, Hofstaedter CE, Zhao C, et al. Optimizing methods and dodging pitfalls in microbiome research. Microbiome. 2017 May 5;5(1):52. 28476139.

Kim HK, Kim JE, Chung J. et al. Surface expression of neutrophil CXCR4 is down-modulated by bacterial endotoxin. Int J Hematol. 2007 Jun;85(5):390-6. 17562613.

Kobayashi Y. The role of chemokines in neutrophil biology. Front Biosci. 2008 Jan 1;13:2400-7. 17981721.

Komine M, Karakawa M, Takekoshi T. et al. Early inflammatory changes in the "perilesional skin" of psoriatic plaques: is there interaction between dendritic cells and keratinocytes? J Invest Dermatol. 2007 Aug;127(8):1915-22. 17446902.

Kong HH, Andersson B, Clavel T. et al. Performing Skin Microbiome Research: A Method to the Madness. J Invest Dermatol. 2017 Mar;137(3):561-568. 28063650.

Kornienko VY, Skin microbiomes: the relationship between microbial community change and disease (literature review), Young Scientist, ISSN 2072-0297, 2015;(10): 477-483. (Rus). elib.

Korotky NG, Peslyak MY. Psoriasis as a consequence of incorporation of beta-streptococci into the microbiocenosis of highly permeable intestines (a pathogenic concept), Vestn Dermatol Venerol, ISSN 0042-4609, 2005;(1): 9-18. (Rus), link1, link2, elib

Kowarsky M, Camunas-Soler J, Kertesz M. et al. Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA. Proc Natl Acad Sci U S A. 2017 Sep 5;114(36):9623-9628. 28830999.

Krueger JG. The immunologic basis for the treatment of psoriasis with new biologic agents.J Am Acad Dermatol. 2002 Jan;46(1):1-23; quiz 23-6. 11756941.

Kubanov AA, Kubanova AA, Karamova AE, Mineyeva AA. Prevalence of genetic risk factors of psoriasis among the population of the Russian Federation. Vestn Dermatol Venerol, ISSN 0042-4609, 2014;(6): 69-76. (Rus). elib.

Lambert S, Hambro CA, Johnston A, Stuart PE, Tsoi LC, Nair RP, Elder JT. Neutrophil Extracellular Traps Induce Human Th17 Cells: Effect of Psoriasis-Associated TRAF3IP2 Genotype. J Invest Dermatol. 2018 Dec 5. pii: S0022-202X(18)32913-0. 30528823.

Lelouvier B, Servant F, Païssé S. et al. Changes in blood microbiota profiles associated with liver fibrosis in obese patients: A pilot analysis. Hepatology. 2016 Dec;64(6):2015-2027. 27639192.

Levitsky E.L. Problem of genetically modified foods safety: a toxicologist's view. BIOTECHNOLOGIA ACTA, 2016, 1, 7-26. elib.

Li D, Li J, Duan Y, Zhou X. Expression of LL37, human beta defensin-2, and CCR6 mRNA in patients with psoriasis vulgaris, J Huazhong Univ Sci Technolog Med Sci. 2004;24(4):404-6. 15587410.

Li G, Yang M, Zhou K. et al. Diversity of Duodenal and Rectal Microbiota in Biopsy Tissues and Luminal Contents in Healthy Volunteers. J Microbiol Biotechnol. 2015 Jul;25(7):1136-45. 25737115.

Li Q, Wang C, Tang C, Zhao X, He Q, Li J. Identification and Characterization of Blood and Neutrophil-Associated Microbiomes in Patients with Severe Acute Pancreatitis Using Next-Generation Sequencing. Front Cell Infect Microbiol. 2018 Jan 23;8:5. 29423379.

Lin A, Loré K. Granulocytes: New Members of the Antigen-Presenting Cell Family. Front Immunol. 2017 Dec 11;8:1781. 29321780.

Lin AM, Rubin CJ, Khandpur R. et al. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. J Immunol.2011 Jul 1;187(1):490-500. 21606249.

Lindau D, Mussard J, Wagner BJ. et al. Primary blood neutrophils express a functional cell surface Toll-like receptor 9. Eur J Immunol. 2013 Aug;43(8):2101-13. 23686399.

Lipp P, Ruhnau J, Lange A, Vogelgesang A, Dressel A, Heckmann M. Less Neutrophil Extracellular Trap Formation in Term Newborns than in Adults. Neonatology. 2017;111(2):182-188. 27884010.

Lluch J, Servant F, Païssé S. et al. The Characterization of Novel Tissue Microbiota Using an Optimized 16S Metagenomic Sequencing Pipeline. PLoS One. 2015 Nov 6;10(11):e0142334. 26544955.

Loesche MA, Farahi K, Capone K. et al. Longitudinal Study of the Psoriasis-Associated Skin Microbiome during Therapy with Ustekinumab in a Randomized Phase 3b Clinical Trial.J Invest Dermatol. 2018 Sep;138(9):1973-1981. 29559344.

Long Y, Zhang Y, Gong Y at al. Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients. Arch Med Res. 2016 Jul;47(5):365-371. 27751370.

Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014 Mar 21;32:227-55. 24655295.

Lowes MA, Russell CB, Martin DA, Towne JE, Krueger JG. The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses. Trends Immunol. 2013 Jan 3. pii: S1471-4906(12)00199-8. 23291100.

Maeshima N, Fernandez RC. Recognition of lipid A variants by the TLR4-MD-2 receptor complex. Front Cell Infect Microbiol. 2013 Feb 12;3:3. 23408095.

Malachowa N, Kobayashi SD, Quinn MT, DeLeo FR. NET Confusion. Front Immunol. 2016 Jun 28;7:259. 27446089.

Malentacchi F, Ciniselli CM, Pazzagli M. et al. Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 2015 Feb 2;440:205-10. 25485853.

Mangul S, Loohuis LMO, Ori A. et al. Total RNA Sequencing reveals microbial communities in human blood and disease specific effects. bioRxiv 057570; http://dx.doi.org/10.1101/057570.

Marotz CA, Sanders JG, Zuniga C. et al. Improving saliva shotgun metagenomics by chemical host DNA depletion. Microbiome. 2018 Feb 27;6(1):42. 29482639.

Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol. 2014;9:181-218. 24050624.

Mazo VK, Gmoshinsky IV, Sablina VV, Zorin SN. Permeability of wall small intestinal for protein antigens in human and laboratory animals. Problems of nutrition. ISSN 0042-8833, 2008;(2): 10-22. (Rus). elib.

McCracken JM, Allen LA. Regulation of Human Neutrophil Apoptosis and Lifespan in Health and Disease. J Cell Death. 2014 May 8;7:15-23. 25278783.

Meisel JS, Hannigan GD, Tyldsley AS. et al. Skin Microbiome Surveys Are Strongly Influenced by Experimental Design. J Invest Dermatol. 2016 May;136(5):947-56. 26829039.

Michalek IM, Loring B, John SM. A systematic review of worldwide epidemiology of psoriasis. J Eur Acad Dermatol Venereol. 2017 Feb;31(2):205-212. 27573025.

Milan AM, Pundir S, Pileggi CA. et al. Comparisons of the Postprandial Inflammatory and Endotoxaemic Responses to Mixed Meals in Young and Older Individuals: A Randomised Trial. Nutrients. 2017 Apr 2;9(4). 28368340.

Mishina OS, Psoriasis morbidity trends in Russia in 2009-2013, Social aspects of population health, ISSN 2071-5021, 2015;(1): 7-15. (Rus). elib

Mitsios A, Arampatzioglou A, Arelaki S, Mitroulis I, Ritis K. NETopathies? Unraveling the Dark Side of Old Diseases through Neutrophils. Front Immunol. 2017 Jan 11;7:678. 28123386.

Munford RS. Endotoxemia-menace, marker, or mistake? J Leukoc Biol. 2016 Oct;100(4):687-698. 27418356.

Munz OH, Sela S, Baker BS et al. Evidence for the presence of bacteria in the blood of psoriasis patients. Arch Dermatol Res. 2010 Sep;302(7):495-8. 20607546.

Nagl M, Kacani L, Müllauer B. et al. Phagocytosis and Killing of Bacteria by Professional Phagocytes and Dendritic Cells. Clin Diagn Lab Immunol. 2002, Nov;9(6):1165-8. 12414745.

Nakatsuji T, Chiang HI, Jiang SB. The microbiome extends to subepidermal compartments of normal skin. Nat Commun. 2013;4:1431. 23385576.

Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol. 2014 Jul;15(7):602-11. 24940954.

Nayfach S, Pollard KS. Toward Accurate and Quantitative Comparative Metagenomics. Cell. 2016 Aug 25;166(5):1103-1116. 27565341.

Niemeyer-van der Kolk T, van der Wall HEC, Balmforth C. et al. A systematic literature review of the human skin microbiome as biomarker for dermatological drug development. Br J Clin Pharmacol. 2018 Oct;84(10):2178-2193. 29877593.

Nikamo P, Lysell J, Ståhle M. Association with Genetic Variants in the IL-23 and NF-κB Pathways Discriminates between Mild and Severe Psoriasis Skin Disease. J Invest Dermatol. 2015 Aug;135(8):1969-1976. 25789703.

b Nickoloff BJ, Xin H, Nestle FO, Qin JZ. The cytokine and chemokine network in psoriasis. Clin Dermatol. 2007 Nov-Dec;25(6):568-73. 18021894.

Numerof RP, Asadullah K. Cytokine and anti-cytokine therapies for psoriasis and atopic dermatitis. BioDrugs. 2006;20(2):93-103. 16626167.

Oehmcke S, Mörgelin M, Herwald H. Activation of the human contact system on neutrophil extracellular traps. J Innate Immun. 2009;1(3):225-30. 20375580.

Oh J, Byrd AL, Deming C. et al. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014 Oct 2;514(7520):59-64. 25279917.

Okubo Y, Oki N, Takeda H, Amaya M. et al. Increased microorganisms DNA levels in peripheral blood monocytes from psoriatic patients using PCR with universal ribosomal RNA primers. J Dermatol. 2002 Sep;29(9):547-55. 12392062.

Opota O, Jaton K, Greub G. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect. 2015 Apr;21(4):323-31. 25686695.

Ortmann W, Kolaczkowska E. Age is the work of art? Impact of neutrophil and organism age on neutrophil extracellular trap formation. Cell Tissue Res. 2018 Mar;371(3):473-488. 29250748.

Ozawa M, Terui T, Tagami H. Localization of IL-8 and complement components in lesional skin of psoriasis vulgaris and pustulosis palmaris et plantaris. Dermatology. 2005;211(3):249-55. 16205070.

Païssé S, Valle C, Servant F. et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion. 2016 May;56(5):1138-47. 26865079.

Panda SK, Kolbeck R, Sanjuan MA. Plasmacytoid dendritic cells in autoimmunity. Curr Opin Immunol. 2017 Feb;44:20-25. 27855321.

Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018 Feb;18(2):134-147. 28990587.

Parfenov AI, Mazo VK, Gmoshinskii IV, Safonova SA, Ekisenina NI. Clinical significance of evaluation of ovalbumin blood level after oral load dose of chicken egg proteins, Russian Journal of Gastroenterology, 1999;(2). (Rus), link

Pashenkov MV, Dagil YA, Pinegin BV. NOD1 and NOD2: Molecular targets in prevention and treatment of infectious diseases. Int Immunopharmacol. 2018 Jan;54:385-400. 29207344.

Peabody MA, Van Rossum T, Lo R, Brinkman FS. Evaluation of shotgun metagenomics sequence classification methods using in silico and in vitro simulated communities. BMC Bioinformatics. 2015 Nov 4;16:363. 26537885.

Perera GK, Di Meglio P, Nestle FO. Psoriasis. Annu. Rev. Pathol. Mech. Dis. 2012. 7:385–422 22054142.

a Peslyak MY. Model of pathogenesis of psoriasis. Part 1. Systemic psoriatic process, Moscow, MYPE, 2012, 84 p., ISBN 9785905504020, link.

b Peslyak MY. Model of pathogenesis of psoriasis. Part 2. Local processes. Moscow, MYPE, 2012, 110 p., ISBN 9785905504044. link.

c Peslyak MY. Psoriasis as skin reaction to systemic psoriatic process SPP. Y-model of pathogenesis. Presentation about theoretical research. 2012, 44 p. DOI: 10.5281/zenodo.1978191.

d Peslyak MY, Gumayunova NG, Nesterov AS, Potaturkina-Nesterova NI, Small intestine microflora at psoriasis. Its possible role in pathogenesis, Abstracts of the 3rd World Psoriasis & Psoriatic Arthritis Conference 2012, Stockholm, Dermatol Ther 2012, 2(10), S12. link, link-R.

a Peslyak MY. Monograph “Metagenomes of blood phagocytes and phagocytes of psoriatic skin. Research project.”, v1.6, Moscow, 2017, 40 p., (rus), DOI: 10.5281/zenodo.1415419.

b Peslyak MY. Presentation “Metagenomes of blood phagocytes and phagocytes of psoriatic skin. Research project.”, v1.6, Moscow, 2017, 77 p., (rus), DOI: 10.5281/zenodo.1439322.

c Peslyak MY, Korotky NG. Research project "Metagenomes of blood phagocytes and phagocytes of psoriatic skin", Presentation. VII Interregional Forum of dermatovenerologists and cosmetologists, IFDC Moscow 2017, 38 p., (Rus), link.

Pieterse E, Rother N, Yanginlar C, Hilbrands LB, van der Vlag J. Neutrophils Discriminate between Lipopolysaccharides of Different Bacterial Sources and Selectively Release Neutrophil Extracellular Traps. Front Immunol. 2016 Nov 4;7:484. 27867387.

Pinegin B, Vorobjeva N, Pinegin V. Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity. Autoimmun Rev. 2015 Jul;14(7):633-40. 25797532.

Poindexter BJ. Immunofluorescence deconvolution microscopy and image reconstruction of human defensins in normal and burned skin. J Burns Wounds. 2005 Apr 25;4:e7. 16921412.

Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev. 2015 Jul;39(4):567-91. 25940667.

Psifidi A, Dovas CI, Bramis G. et al. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. PLoS One. 2015 Jan 30;10(1):e0115960. 25635817.

Puri P, Liangpunsakul S, Christensen JE, et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. Hepatology. 2018 Apr;67(4):1284-1302. 29083504.

Qian Y, Yang X, Xu S. et al. Detection of Microbial 16S rRNA Gene in the Blood of Patients With Parkinson's Disease. Front Aging Neurosci. 2018 May 24;10:156. 29881345.

Rademaker M, Agnew K, Anagnostou N. et al. Psoriasis and infection. A clinical practice narrative. Australas J Dermatol. Epub 2018 Aug 5. 30079566.

Ramírez-Boscá A, Navarro-López V, Martínez-Andrés A. et al. Identification of Bacterial DNA in the Peripheral Blood of Patients With Active Psoriasis. JAMA Dermatol. 2015 Jun;151(6):670-1. 25760018.

Ranjan R, Rani A, Metwally A. et al. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun. 2016 Jan 22;469(4):967-77. 26718401.

Rankin SM. The bone marrow: a site of neutrophil clearance. J Leukoc Biol. 2010 Aug;88(2):241-51. 20483920.

Reddy RC, Standiford TJ. Effects of sepsis on neutrophil chemotaxis. Curr Opin Hematol. 2010 Jan;17(1):18-24. 19864946.

Reich K, Papp KA, Matheson RT. et al. Evidence that a neutrophil-keratinocyte crosstalk is an early target of IL-17A inhibition in psoriasis. Exp Dermatol. 2015 Jul;24(7):529-35. 25828362.

Rizzi A, Raddadi N, Sorlini C. et al. The stability and degradation of dietary DNA in the gastrointestinal tract of mammals: implications for horizontal gene transfer and the biosafety of GMOs. Crit Rev Food Sci Nutr. 2012;52(2):142-61. 22059960.

Rohrl J, Yang D, Oppenheim JJ, Hehlgans T. Human beta-defensin 2 and 3 and their mouse orthologs induce chemotaxis through interaction with CCR2. J Immunol. 2010 Jun 15;184(12):6688-94. 20483750.

Rudkovskaya ZV, Clinical and laboratory monitoring of efficiency of application of method of interval normobaric hypoxia in complex treatment of psoriasis in children. Dissertation, Moscow, 2003, 137 p., (Rus).

Sabat R, Philipp S, Höflich C. et al. Immunopathogenesis of psoriasis. Exp Dermatol. 2007 Oct;16(10):779-98. 17845210.

Sangaletti S, Tripodo C, Chiodoni C. et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood. 2012 Oct 11;120(15):3007-18. 22932797.

Sato J, Kanazawa A, Ikeda F. et al. Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. Diabetes Care. 2014 Aug;37(8):2343-50. 24824547.

Sekine K, Toida T, Saito M. et al. A New Morphologically Characterized Cell Wall Preparation (Whole Peptidoglycan) from Bifidobacterium infantis with a Higher Efficacy on the Regression of an Established Tumor in Mice. Cancer Res. 1985 Mar;45(3):1300-7. 3971375.

Schuster S, Hurrell B, Tacchini-Cottier F. Crosstalk between neutrophils and dendritic cells: a context-dependent process. J Leukoc Biol. 2013 Oct;94(4):671-5. 23250891.

Schon MP, Broekaert SM, Erpenbeck L. Sexy again: The renaissance of neutrophils in psoriasis. Exp Dermatol. 2017 Apr;26(4):305-311. 27194625.

Schon MP, Erpenbeck L. The interleukin-23/interleukin-17 Axis Links Adaptive and innate immunity in Psoriasis. Front Immunol. 2018 Jun 15;9:1323. 29963046.

Schonrich G, Raftery MJ. Neutrophil Extracellular Traps Go Viral. Front Immunol. 2016 Sep 19;7:366. 27698656.

Sharpton TJ. An introduction to the analysis of shotgun metagenomic data. Front Plant Sci. 2014 Jun 16;5:209. 24982662.

Shen XF, Cao K, Jiang JP, Guan WX1, Du JF. Neutrophil dysregulation during sepsis: an overview and update. J Cell Mol Med. 2017 Sep;21(9):1687-1697. 28244690.

Shiryaeva YuA. Malabsorption syndrome at psoriatics. Dissertation, Krasnoyarsk, 2007, 150 p. (Rus)

Skrzeczynska-Moncznik J, Wlodarczyk A, Zabieglo K. et al. Secretory Leukocyte Proteinase Inhibitor-Competent DNA Deposits Are Potent Stimulators of Plasmacytoid Dendritic Cells: Implication for Psoriasis. J Immunol. 2012 Aug 15;189(4):1611-7. 22786767.

Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol. 2010 Jun;10(6):427-39. 20498669.

Song Y, Giske CG, Gille-Johnson P. et al. Nuclease-assisted suppression of human DNA background in sepsis. PLoS One. 2014 Jul 30;9(7):e103610. 25076135.

Souto FO, Alves-Filho JC, Turato WM. et al. Essential role of CCR2 in neutrophil tissue infiltration and multiple organ dysfunction in sepsis. Am J Respir Crit Care Med. 2011 Jan 15;183(2):234-42. 20732989.

Sozzani S. Dendritic cell trafficking: more than just chemokines. Cytokine Growth Factor Rev. 2005 Dec;16(6):581-92. 15963754.

Spisák S, Solymosi N, Ittzés P. et al. Complete genes may pass from food to human blood. PLoS One. 2013 Jul 30;8(7):e69805. 23936105.

Statnikov A, Alekseyenko AV, Li Z. et al. Microbiomic signatures of psoriasis: feasibility and methodology comparison.Sci Rep. 2013 Sep 10;3:2620. 24018484.

Stenina MA, Kulagin VI, Rudkovskaya ZV et al, Role of disturbances of intestine barrier function in pathogenesis of psoriasis in children, Russian Journal of Skin and Sexually Transmitted Diseases, ISSN 1560-9588. 2003;(2):20-23. (Rus), elib.

Stevenson M, Pandor A, Martyn-St James M, et al. Sepsis: the LightCycler SeptiFast Test MGRADE®, SepsiTest™ and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi - a systematic review and economic evaluation. Health Technol Assess. 2016 Jun;20(46):1-246. 27355222.

Suzuki N, Kamataki A, Yamaki J, Homma Y. Characterization of circulating DNA in healthy human plasma. Clin Chim Acta. 2008 Jan;387(1-2):55-8. 17916343.

Tacke F, Ginhoux F, Jakubzick C, van Rooijen N, Merad M, Randolph GJ. Immature monocytes acquire antigens from other cells in the bone marrow and present them to T cells after maturing in the periphery. J Exp Med. 2006 Mar 20;203(3):583-97. 16492803.

Takemoto A, Cho O, Morohoshi Y, Sugita T, Muto M. Molecular characterization of the skin fungal microbiome in patients with psoriasis. J Dermatol. 2015;42(2):166–70. 25510344.

Tan B, Ng C, Nshimyimana JP. et al. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. Front Microbiol. 2015 Sep 25;6:1027. 26441948.

Tanes CE. Psoriasis: A Study of the Skin Transcriptome and Microbiome. 2015. Drexel University, 107 p. link.

Tett A, Pasolli E, Farina S. et al. Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. NPJ Biofilms Microbiomes. 2017 Jun 22;3:14. 28649415.

Terui T, Ozawa M, Tagami H. Role of neutrophils in induction of acute inflammation in T-cell-mediated immune dermatosis, psoriasis: a neutrophil-associated inflammation-boosting loop. Exp Dermatol. 2000 Feb;9(1):1-10. 10688368.

Tonel G, Conrad C. Interplay between keratinocytes and immune cells - Recent insights into psoriasis pathogenesis. Int J Biochem Cell Biol. 2009 May;41(5):963-8. 19027868.

Truong DT, Franzosa EA, Tickle TL. et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods. 2015 Oct;12(10):902-3. 26418763.

Tsoi LC, Stuart PE, Tian C. et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. Nat Commun. 2017 May 24;8:15382. 28537254.

Tyaht AV. Functional analysis of human intestinal metagenome. Dissertation. Moscow, 2014, 131 p., (Rus), link.

Uhl B, Vadlau Y, Zuchtriegel G. et al. Aged neutrophils contribute to the first line of defense in the acute inflammatory response. Blood. 2016 Nov 10;128(19):2327-2337. 27609642.

Urosevic M, Dummer R, Conrad C. et al. Disease-Independent Skin Recruitment and Activation of Plasmacytoid Predendritic Cells Following Imiquimod Treatment. J Natl Cancer Inst. 2005 Aug 3;97(15):1143-53. 16077073.

Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A. Psoriasis - as an autoimmune disease caused by molecular mimicry. Trends Immunol. 2009 Oct;30(10):494-501. 19781993.

van den Berg JM1, Weyer S, Weening JJ, Roos D, Kuijpers TW. Divergent effects of tumor necrosis factor alpha on apoptosis of human neutrophils. J Leukoc Biol. 2001 Mar;69(3):467-73. 11261795.

van de Kerkhof P.C.M. The evolution of the psoriatic lesion. British Journal of Dermatology 2007, 157, pp4–15 17578434.

Vanderpuye-Orgle J, Zhao Y, Lu J. et al. Evaluating the economic burden of psoriasis in the United States. J Am Acad Dermatol. 2015 Jun;72(6):961-7.e5. 25882886.

Vestergaard C, Just H, Baumgartner Nielsen J et al. Expression of CCR2 on monocytes and macrophages in chronically inflamed skin in atopic dermatitis and psoriasis. Acta Derm Venereol. 2004;84(5):353-8. 15370700.

Vetrovsky T, Baldrian P. The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. PLoS One. 2013;8(2):e57923. 23460914.

Vincent AT, Derome N, Boyle B. et al. Next-generation sequencing (NGS) in the microbiological world: How to make the most of your money. J Microbiol Methods. 2017 Jul;138:60-71. 26995332.

Vojdani A. For the assessment of intestinal permeability, size matters. Altern Ther Health Med. 2013 Jan-Feb;19(1):12-24. 23341423.

Vollmer W1, Seligman SJ. Architecture of peptidoglycan: more data and more models. Trends Microbiol. 2010 Feb;18(2):59-66. 20060721.

Vorobjeva NV, Pinegin BV. Neutrophil extracellular traps: mechanisms of formation and their role in health and disease. ISSN 00062979, Biochemistry (Moscow), 2014;(12): 1286-1296. 25716722, elib.

Wang Q, Vasey FB, Mahfood JP, Valeriano J, Kanik KS, Anderson BE, Bridgeford PH. V2 regions of 16S ribosomal RNA used as a molecular marker for the species identification of streptococci in peripheral blood and synovial fluid from patients with psoriatic arthritis. Arthritis Rheum. 1999 Oct;42(10):2055-9. 10524676.

a Wang J, Arase H. Regulation of immune responses by neutrophils. Ann N Y Acad Sci. 2014 Jun;1319:66-81. 24850053.

b Wang XN, McGovern N, Gunawan M. et al. A Three-Dimensional Atlas of Human Dermal Leukocytes, Lymphatics, and Blood Vessels. J Invest Dermatol. 2014 Apr;134(4):965-74. 24352044.

Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol. 2014 Mar 3;15(3):R46. 24580807.

Wright W.F, Overman S.B, Ribes J.A. (1–3)-beta-D-Glucan Assay: A Review of its Laboratory and Clinical Application. Laboratory Medicine, Volume 42, Issue 11, 1 November 2011, Pages 679–685, link.

Yan D, Issa N, Afifi L. et al. The Role of the Skin and Gut Microbiome in Psoriatic Disease. Curr Derm Rep (2017). doi:10.1007/s13671-017-0178-5.

Yang D, Chertov O, Bykovskaia SN. et al. beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6, Science. 1999 Oct 15;286(5439):525-8. 10521347.

Yin X, Cheng H, Lin Y. et al. A Weighted Polygenic Risk Score Using 14 Known Susceptibility Variants to Estimate Risk and Age Onset of Psoriasis in Han Chinese. PLoS One. 2015 May 1;10(5):e0125369. 25933357.

Yigit E, Feehery GR, Langhorst BW. et al. A Microbiome DNA Enrichment Method for Next-Generation Sequencing Sample Preparation.Curr Protoc Mol Biol. 2016 Jul 1;115:7.26.1-7.26.14. 27366894.

a Zaba LC, Fuentes-Duculan J, Eungdamrong NJ et al. Psoriasis Is Characterized by Accumulation of Immunostimulatory and Th1/Th17 Cell-Polarizing Myeloid Dendritic Cells. J Invest Dermatol. 2009 Jan;129(1):79-88. 18633443.

Zhou L, Pollard AJ. A novel method of selective removal of human DNA improves PCR sensitivity for detection of Salmonella Typhi in blood samples. BMC Infect Dis. 2012 Jul 27;12:164. 22839649.

Znamenskaya LF, Melekhina LYe, Bogdanova YeV, Mineyeva AA., Psoriasis incidence and prevalence in the Russian Federation. Vestn Dermatol Venerol, ISSN 0042-4609, 2012;(5): 20-29, (Rus). elib