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Mini Review

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Translational applications of diagnostics of infectious diseases using infectomics approaches in clinical settings

Abstract

Modern molecular and biochemical technologies like polymerase chain reaction (PCR), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), or microarray, evolved the diagnostic strategies of infectious diseases and changed the routine workflow in clinical microbiology laboratories. However, we still cannot identify causative organisms of many infectious diseases, like febrile neutropenia, sepsis, pneumonia, systemic mycoses and culture-negative endocarditis, in critically ill patients even though using such molecular techniques, and consequently we use antimicrobials or antifungals empirically for these cases without any evidence of pathogens specified. Now we need to find out the alternative way for diagnostics of infectious diseases. In the post-genomics era, undiagnosed infectious diseases would be analyzed by comprehensive data mining and hierarchical algorithm obtained from perspective infectome that analyzes host-pathogen-microbiome interactions. Accordingly we require a new translational application in clinical settings from this infectomics. Detecting pathogen-specific or infected host-derived volatile organic compounds is one of the good answers because it is non-invasive, easily performed, rapid, inexpensive, and available for point-of-care testing for diagnosis of infectious diseases. This mini-review focuses unmet needs of diagnostics of infectious diseases in clinical settings and impact of alternative diagnostic ways using infectomics approaches and their clinical implications.

Keywords: infectomics, proteome, metabolome, volatile organic compounds, point-of-care testing, clinical settings

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Abbreviations: PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; NGS, next generation sequence; DNA, deoxyribonucleic acid; CRP, c-reactive protein; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HUPO, human proteome organization; PPP, plasma proteome project; PSI, proteomics standards initiative; FDA, food and drug administration; TRAIL, tnf-related apoptosis-inducing ligand; IP-10, interferon gamma induced protein-10; UHPLC-ESI-Q-TOF-MS, ultrahigh performance liquid chromatography-electro spray ionization-quadruple time of flight-mas spectrometry; NMR, nuclear magnetic resonance; VOCs, volatile organic compounds; e-noses, electronic noses; EIA, enzyme immune-assay; EORTC, european organization for research and treatment of cancer/invasive fungal infections cooperative group

Introduction

Culture-independent modern molecular technologies like polymerase chain reaction (PCR), microarray, or matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) provided drastic evolution for diagnostics of infectious diseases.[1–6] These novel approaches allowed us to shorten the time to complete final reports several hours to days in the clinical microbiology laboratories, compared with conventional culture-based methods.[2–4] Although it is unlikely that these methods entirely replace the conventional culture-based approach because of importance of phenotypic characterization, typing, antimicrobial susceptibility testing, and making stocks for public health resources of microbes, a rapid, reliable, accurate and easily performed method for detection of microorganism contributes to improve patient care including management of individuals and infection control.[5,6] As early diagnosis of infectious diseases leads appropriate use of target-oriented antimicrobials and antifungals, unnecessary selection of drug-resistant bacteria or fungi can be prevented. However, even though these molecular techniques are generally used, we often encounter some cases that are not able to differentiate infectious diseases from other diseases like fever of unknown origins. The next generation sequence (NGS) technology brought us another impact for undiagnosed infectious diseases because its high-throughput sequence can elucidate all the nucleic acid in specimens whether physicians suppose expected microbes or not.[5–7] If we can collect adequate aseptic specimens such as whole blood, plasma/serum, cerebrospinal fluid, resected tissues or pleural effusion, it is easy to interpret the results of NGS by excluding host DNAs. However, commercial based NGS systems for diagnosis of infectious diseases are not available and the sensitivity of NGS is not higher than that of conventional PCR because of lesser amounts of nucleic acids other than human origin.[7] Therefore, we need to discover the alternative way for diagnostics of infectious diseases. In the post-genomic era, huge database of omics like proteome, metabolome, or transcriptome analysis has been accumulated and their profiles of patient samples may reveal specific diagnostic biomarkers of both host and microbe origins. This mini-review focuses the alternative diagnostics of infectious diseases using new omics approaches beyond genomics in clinical settings.

Approaches using infectomics for diagnostics of infectious diseases

In the era of post-genome that is symbolized by NGS, we have big data of systematic biological information complex such as transcriptome as systematic gene expression, proteome, or metabollome. The term of omics is defined by a comprehensive science system managed and analyzed these large biological data, as transcriptomics, proteomics, or metabolomics.[8] The advent of the omics allows us to evolve the paradigm shift of medical science and to pave the way to understand molecular and cellular processes of various diseases, such as cardiovascular diseases, metabolic disorders, endocrine diseases, neoplasms as well as infectious diseases.[8] Fontana et al.,[8] advocated "infectome" of the omics paradigm for host-microbe interactions.[8] We would show the schematic representation of modified "infectomics" as better understanding infectious diseases comprehensively (Figure 1). The host-pathogen interaction is a major event in infectious diseases. The pathogen triggers the host defense systems based on innate and acquired immunity. The virulence factors produced by pathogens, such as various toxins, metabolites, or adhesins, help to promote infection to host cells. Considering infectious diseases, the third components as habitat microorganisms in hosts play an important role.[8,9] We have myriad bacteria, fungi even though viruses as commensals on skin, oral-pharyngeal-gastrointestinal or urogenital mucosa.[8,9] These comprise normal flora as symbiotic (merits for humans as host defense, or supply of nutrients like vitamins) or parasitic habits. This microbial ecosystem allows us to maintain human health and to prevent some diseases.[10,11] In 2007, the national institute of health (NIH) began the Human Microbiome Project (HMP) to elucidate the core microbiome in healthy subjects and the relationship between alterations of the microbiome and various diseases.[12] These three components interfere each other and infectious diseases occur when their balance is altered. Accordingly, the term of "infectomics" is defined by comprehensive analysis of these three component complexities for understanding infectious diseases. Clinical intervention of infectomics will require artificial intelligence because of integration between big trans-omics data and diagnosis process in physicians.

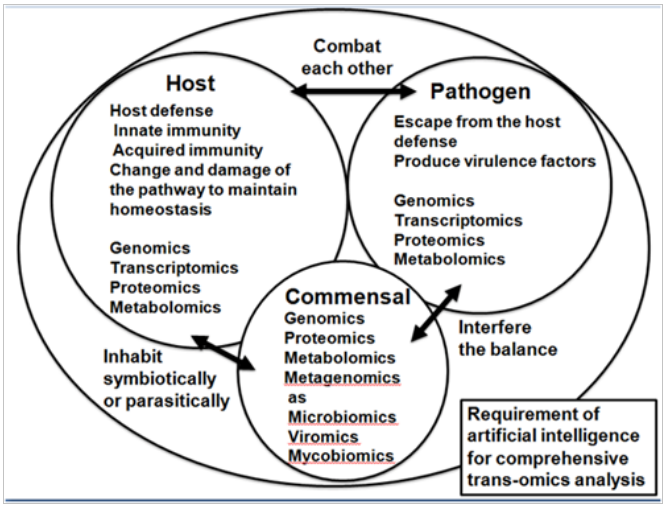

Figure 1 Schematic representation of infectomics.

In the comparative differential proteomics strategy, comprehensive screening is performed for specimens (e.g. serum/plasma) in patients to identify candidates that expressed in response to specific infections as compared with healthy controls or resembled diseases.[13] Selecting proteins combinations and appropriate cohorts are constructed for validation by immunoassays or liquid chromatography-mass spectrometry (LC-MS) in each protein targets. Human Proteome Organization (HUPO) Plasma Proteome Project (PPP) and Proteomics Standards Initiative (PSI) that has been started since 2002 provide useful bioinformatics database for systematic screening target proteins.[13] Many proteins such as serum amyloid A, hemopexin, apolipoprotein A-I, haptoglobin, prostaglandin H2, b-thromboglobin, a1-acid glycoprotein, a-1-antitrypsin, and retinol-binding protein-4 are up-regulated or down-regulated in dependent on various infectious diseases.[13] Unfortunately, many of them are altered in the same way in various infectious diseases regardless of causative organisms. There are only a few single biomarkers with real clinical applicability and Food and Drug Administration (FDA) approval.[13] Oved et al.,[14] reported that TNF-related apoptosis-inducing ligand (TRAIL) from 600 protein candidates was consistently up regulated in viral infected patients.[14] The best combination to differentiate bacterial and viral infections is TRAIL, interferon gamma induced protein-10 (IP-10) and CRP.[14] Immunoproteomics approach is another way to screen new pathogen-specific biomarkers of infectious diseases. Systematic large numbers of proteins of pathogens are screened with antibodies from patients diagnosed the specific infectious disease using high-throughput methods such as microarray. There have been several reports for diagnostics of infectious diseases caused by *Candida*.[15,16] and *Trypanosoma*.[17] This approach is also useful for selecting vaccine targets.[18]

Analyzing differential profiles of relative small molecules between targeted infectious diseases and healthy controls is another approach for identification of disease-specific microbes. Several compounds are derived from host reactions due to activation of infection-associated metabolic pathways and also from microbial processing of host metabolites. Large numbers of metabolites are differentiated by LC-MS, ultrahigh performance liquid chromatography-electrospray ionization-quadruple time of flight-mas spectrometry (UHPLC-ESI-Q-TOF-MS), or nuclear magnetic resonance (NMR) spectroscopy.[19–21] Metabolomics can successfully discover several biomarkers for human infectious diseases including diagnostic approach for malaria,[22] tuberculosis,[20,21] schistosomiasis,[23] Lyme disease,[24] *Escherichia coli* urinary tract infection,[25] and bacteremic sepsis.[19] Although some transcriptome analyses are useful for better understanding both the change of cellular pathway of specific infectious diseases and the shift of virulence traits in pathogens,[26,27] it is far away to introduce them into routine laboratory works. There are several reports that the microbiota composition of gut, urogenital tract, or oropharynx plays a protective or inversely promotive role of various infectious diseases.[28–31]

What do we need using infectomics in clinical settings?

Infectious diseases physicians benefit advancement of molecular technology such as PCR or MALDI-TOF MS for diagnosis and management of infectious diseases. However, we often encounter undiagnosed cases suspected infection even though molecular methods are available. Table 1 showed such clinical situations required alternative applications in diagnostics of infectious diseases. Discrimination between infection and non-infection is sometimes problematic. Febrile neutropenia mainly occur in patients with hematological malignancy or solid cancer under chemotherapy. Since most of these patients receive preventable administration of antimicrobial or antifungal agents, usefulness of conventional microbiology tests such as blood culture is limited. Culture-negative endocarditis represents 2.5-31% of cases of endocarditis including caused by fastidious or uncultured microorganisms such as *Bartonella, Coxiella, Mycoplasma, Histoplasma,* or *Troperyma whipplei*, as causative pathogens, with blood culture sterilized by

antimicrobial treatment before diagnosis of endocarditis, and non-infection endocarditis associated with systemic diseases such as lupus or Behçet disease.[32] For abnormal imaging findings in X-ray, computed tomography (CT) scan, or magnetic resonance imaging (MRI), differentiation among infection, tumor, and granuloma is required. We also have many cases suspected infection because of acute onset and progression, positive acute inflammatory signs but without evidence of specific pathogens. Empirical treatments of broad-range antimicrobials or antifungals are frequently started for these cases before definite diagnosis to be aware of spreads of multi-drug resistant microorganisms. Non-aseptic specimens possibly contaminated with normal flora such as sputum, urine, or pharyngeal swab, yield commensal bacteria or fungi. Diagnosis of systemic invasive candidosis is sometimes problematic like cases with systemic inflammatory signs and positive cultures from several specimens other than blood cultures. The predictive biomarkers of invasiveness by such organisms are required. Another problem of diagnostics of infectious diseases in clinical settings is collection of specimens. To diagnose of pulmonary infections, ideal lung specimens collected by open-trans bronchial lung biopsy or bronchial mucosa biopsy are more invasive than exhaled sputum usually contaminated with oro-pharyngeal commensals. Resected valve tissues are required for broad-range PCR detection of causative organisms in culture-negative endocarditis.[33,34] Non-invasive procedures to collect clinical samples are suitable for small children and critically ill patients in intensive care units.

Table 1 Requirement of new diagnostics of infectious diseases in clinical situations

Category of diagnosis required	Clinical situations supposed
Infection vs. non-infection	Fever of unknown origins
	Febrile neutropenia
	Abnormal imaging finding in X-ray, CT, MRI, etc.
	Sepsis, septic shock
	Acute respiratory distress syndrome (ARDS)
	Disseminated intravascular coagulation (DIC)
	Fever or any inflammatory signs without response to antimicrobials
	Culture-negative endocarditis, pericarditis, aneurysm
	Arthritis of unknown etiology
	Sudden death with unknown etiology
	Any disease status supposed infection
Bacterial vs. viral	Febrile neutropenia suspected infection
Bacterial vs. fungal	Abnormal imaging finding in X-ray, CT, MRI, etc. associated with infection suspected
Viral vs. fungal	Sepsis, septic shock suspected infection without evidence of specific pathogens
	ARDS suspected infection without evidence of specific pathogens
	DIC suspected infection without evidence of specific pathogens
Parasitic vs. bacterial	Fever or any inflammatory signs suspected infection without response to antibiotics
	Culture-negative endocarditis, pericarditis, infected aneurysm suspected
	Pneumonia, pulmonary infections without evidence of specific pathogens
	Generalized rash with fever without evidence of specific pathogens
	Culture-negative spontaneous bacterial peritonitis
	Aseptic meningitis
	Deep abscess without evidence of specific pathogens
	Arthritis associated with infection suspected
	Pelvic inflammatory disease without evidence of specific pathogens
	Surgical site infection without evidence of specific pathogens
	Gastroenteritis, especially traveler's diarrhea
	Fever in returning travelers without evidence of specific pathogens
	Any infections suspected without evidence of specific pathogens

Table Continued....

Category of diagnosis required	Clinical situations supposed
Invasive vs. non-invasive	Invasive candidosis in a patient associated with positive culture from contaminated specimens
Contaminated vs. clinically significant	Bacteriuria, candiduria
	Positive blood or aseptic specimen culture of microbes usually contaminated such as coagulase-negative *staphylococci*
Specified pathogen	Systemic mycoses with some evidence, e.g. positive b-D-glucan
	Granuloma or any tissues suspected infections pathologically without any evidence of pathogens

Hereby, we need to translate infectomics in basic research to clinical applications to order to solve these problems above-mentioned for diagnostics of infectious diseases. Applications that fulfill rapidness, easy handle, low costs, unnecessary of special equipments, reliability, and easy interpretation of results, should be available in routine clinical laboratory workflows. The point-of-care testing (POCT) is the ultimate way for this object.

Translational applications available in clinical settings; detection of volatile organ compounds (VOCs) in infectious diseases

One of the good answers for translational infectome applications for diagnostics of infectious diseases in clinical settings is detecting volatile organic compounds (VOCs). Since Hippocrates suggested that pouring sputum on hot coals was useful to diagnose tuberculosis, discerning a patient's disease-specific odor had been an important skill of diagnostics of infectious diseases in ancient physicians.[35,36] Recently, trained animals have been able to detect various types of cancers by disease-specific smells indicated VOCs as well as infectious diseases.[35,36] The various VOCs are detectable in exhaled breath, headspace of urine, feces, and sweat collected from patients with infectious diseases.[35,36] Microorganisms produce their own VOCs *in vitro*, indicated pathogen-specific biomarker candidates. Clinical specimens contain disease-specific VOCs composed of complexes of pathogen-produced and host-derived metabolites.[35,36] The plausible sources of VOCs are also derived from microbiota in gastrointestinal tracts because gut is a large fermenting chamber with production of various metabolites.[35] Thereby, analysis of VOCs reflects three components of infectome in Figure 1. The animal sense of VOCs can replace gas chromatography-mass spectrometry (GC-MS) and the further developed equipments such as proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS), or secondary electro spray ionization mass spectrometry (SESI-MS) that are able to identify wide range of VOCs reproducibly.[35–38] Although these newly developed analytical devices can identify VOCs without pre-concentration or separation procedures, there are several disadvantages used in clinical laboratories, such as immobile system, time-consuming, high costs, or technical training required.[35,36] The new instruments including ion mobility spectrometry (IMS), electronic noses (e-noses) that resemble mammalian olfactory system like sensor-neural networks, have been successfully for convenient detection of VOCs on POCT.[35,36] The e-noses are composed of an array of non-specific electronic chemical sensors and pattern-recognition system of VOCs, and are non-invasive, portable, rapid, inexpensive, easy to use, and no requirement of special training.[35,36] The majority of recent studies measuring VOCs are about diagnosis of respiratory infections using exhaled breath as clinical samples.[36,38] Although literatures for detection of causative-organism specific VOCs in infectious diseases are still limited and most of them showed low numbers of samples, clinical trials with specific organisms by detecting VOCs are summarized in Table 2. Measuring VOC biomarkers were also useful for diagnosis of ventilator-associated pneumonia, chronic obstructive pulmonary disease (COPD) exacerbations, malaria, and urinary tract infections.[59–64] Further systematic expansion of database of pathogen-specific VOCs, disease-specific VOCs, and commensal VOCs would be required for more accurate and reliable diagnostics of infectious diseases.[65]

Table 2 Diagnosis of infectious diseases by specific microorganisms by VOCs profiles

Disease	Causative organism	Specimen	Subject (No.)	Control (No.)	Detection Method	POCT	Sensitivity	Specificity	References
Colitis	*Clostridium difficile*	Feces	Culture-positive (6)	Healthy control (6)	GC-MS	not available	0.83	0.97	39
		Feces	Culture-positive (77)	Culture-negative (23)	GC-MS	not available	0.83	1	40
		Feces	*C. difficile*-toxin EIA test positive (50)	EIA negative (50)	SMCC with MOS sensora	possible	0.85	0.8	41
		Feces	*C. difficile*-PCR positive (20)	PCR-negative (53)	electric nose	possible	0.8	0.85	42
		Feces	Culture, toxin-positive (48)	Toxin-negative (84)	FAIMSb	possible	0.923	0.86	43
	Campylobacter jejuni	Feces	Culture-positive (5)	Healthy control (6)	GC-MS	not available	1	0.92	39
Gastritis	*Helicobacter pylori*	Exhaled breath	Culture-positive patients (6)	Healthy control (23)	GC-MS	not available	0.67	1	44

Table Continued....

Disease	Causative organism	Specimen	Subject (No.)	Control (No.)	Detection Method	POCT	Sensitivity	Specificity	References
Tuberculosis	*Mycobacterium tuberculosis*	Sputum	Culture-positive (55)	Culture-negative (79)	electric nose	possible	0.91	0.89	45
		Sputum	Smear-negative, culture-positive (56)	Smear-negative, culture-negative (228)	electronic nose (EN Rob)	possible	0.68	0.69	46
		Sputum	Smear-negative, culture-positive (80)	Smear-negative, culture-negative (243)	electronic nose (EN Water)	possible	0.75	0.67	46
		Exhaled breath	Culture-positive (23)	Culture-negative (19)	GC-MS	not available	0.96	0.79	47
		Exhaled breath	Tuberculosis suspected patients (226)		GC-MS	not available	0.84	0.65	48
		Exhaled breath	Smear or culture-positive (130)	healthy control (121)	GC/surface acoustic wave detector	possible	0.72	0.72	49
		Exhaled breath	Culture-positive (71)	Culture-negative (100)	GC-MS	not available	0.41	0.91	50
		Exhaled breath	Culture-positive (34)	Culture-negative (114)	electric nose	available	0.77	0.87	51
		Exhaled breath	Probable aspergillosis with EORTC criteria (5)	No aspergillosis (6)	electric nose	available	1	0.833	52
Invasive aspergillosis	*Aspergillus fumigatus*	Exhaled breath	Invasive aspergillosis (34)	Other pneumonia (30)	GC-MS	not available	0.94	0.93	53
		Exhaled breath	A. fumigatus colonization in cystic fibrosis patients (9)	No A. fumigatus in cyctic fibrosis patients (18)	electric nose	available	0.78	0.94	54
Lung infection in cystic fibrosis, bronchiectasis patients	*Pseudomonas aeruginosa*	Sputum	Culture-positive (32)	Culture-negative (40)	GC-MS	not available	0.91	0.88	55
		Sputum	Culture-positive (9)	Culture-negative (19)	GC-MS	not available	1	0.67	56
		Exhaled breath	Lung infection in cystic fibrosis patients (48)	Healthy control (57)	GC-MS	not available	1	1	57
		Exhaled breath	Lung infection in cystic fibrosis patients (44)	Cystic fibrosis patients without *P. aeruginosa* infection (29)	SIFT-MSc	not available	0.83	0.71	58

[a]SMCC with MOS, short multi-capillary chromatography column with metal oxide semiconductor sensor.

[b]FAIMS, field asymmetric ion mobility spectrometry.

[c]SIFT-MS, selected ion-flow tube mass spectrometry.

Conclusion

Even though culture-independent molecular technologies are available, we have many diagnostic problems in infectious diseases in various clinical situations. Infectomics defined by comprehensive analysis of host-pathogen-commensal complexities is useful for better understanding infectious diseases, developing new antimicrobials and vaccines. Translational applications from infectomics like detecting volatile organic compounds are responsive to the unmet needs of diagnostics of infectious diseases in clinical settings.

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Conflict of interest

The author declares no conflict of interest.

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