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Review

Assessment of immune status using blood transcriptomics and potential implications for global health

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ABSTRACT

The immune system plays a key role in health maintenance and pathogenesis of a wide range of diseases. Leukocytes that are present in the blood convey valuable information about the status of the immune system. Blood transcriptomics, which consists in profiling blood transcript abundance on genome-wide scales, has gained in popularity over the past several years. Indeed, practicality and simplicity largely makes up for what this approach may lack in terms of cell population-level resolution. An extensive survey of the literature reveals increasingly widespread use across virtually all fields of medicine as well as across a number of different animal species, including model organisms but also animals of economical importance. Dissemination across such a wide range of disciplines holds the promise of adding a new perspective, breadth or context, to the considerable depth afforded by whole genome profiling of blood transcript abundance. Indeed, it is only through such contextualization that a truly global perspective will be gained from the use of systems approaches. Also discussed are opportunities that may arise for the fields of immunology and medicine from using blood transcriptomics as a common denominator for developing interactions and cooperation across fields of research that have traditionally been and largely remain compartmentalized. Finally, an argument is made for building immunology research capacity using blood transcriptomics platforms in low-resource and high-disease burden settings.

1. Introduction to systems approaches

The use of systems approaches is transforming the biomedical research landscape by enabling the comprehensive molecular profiling of cells and tissues obtained from human subjects in health and disease. Systems approaches leverage high throughput technologies to measure all the parameters that can be measured in a given biological system (often referred to as "omics" technologies). Indeed, as a result, and in contrast with the more "traditional" assays, investigators do not need to choose the parameters that will be measured as it will by default simply be "everything", and it is in that sense that these approaches are inherently unbiased. Genomics is probably the most popular omics approach and has benefited from swift technological advances that allow measurements of constitutive elements of a system on a genome-wide scale in a single assay (sequence, transcript abundance, methylation sites, transcription binding sites, etc). Transcriptome profiling constitutes a genomic approach since it consists in measuring transcript abundance in a sample on a genome-wide scale, and relies on detection or sequencing of oligonucleotide sequences that are present in a sample. Other Omics approaches rely on technologies that tend to be more costly and challenging to implement (e.g. proteomics) or that rely on a multiplicity of assays (e.g. metabolomics) [1]. Indeed a wide range of molecular profiling platforms are available to immunologists, that despite having not yet truly reached system levels, allow for molecular and cellular profiles on increasingly large scales – (for instance multiplex protein profiling or multi-parameter flow cytometry) [2,3]. Systems Immunology encompasses the use of such systems or large-scale approaches in the context of immunological studies. Indeed interdependence exists amongst elements within a given system but also across elements constitutive of the different systems (e.g. between genome sequences, transcriptomes, proteomes, microbiomes as well as cellular profiles). Thus it is more and more common for immunological studies to incorporate multiple systems-scale or large-scale profiling approaches [4,5]. Notably, while genomic profiling capabilities have been available for the past 15 years, technology platforms have continued to gain in sophistication – while, inevitably, analytic pipelines have concurrently gained in complexity. And each time a new study is implemented the abundance, complexity and heterogeneity of the data it

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produces increases in comparison with the previous study. Taken together the data thus being generated has become referred to as biomedical "Big Data", with investigators indeed having increasingly to contend with Big Data's canonical 3 Vs, which stand for Volume (scale), Variety (heterogeneity) and Velocity (speed at which the data can be accessed and interrogated). Indeed capturing, storing and integrating all of this data within a given study, and especially across studies – i.e. collective biomedical Big Data – will challenge even the most advanced information technology solutions within well-resourced research organizations.

2. Adding breadth to the depth of systems-scale investigations

This review will focus on only one of several systems or large-scale profiling approaches available as part of the systems immunology armamentarium: blood transcriptome profiling. Other articles in this issue cover the use of transcriptome profiling in isolated immune cell populations. Here I will examine the use of blood transcriptomics technology, an approach that consists in measuring abundance of RNA in peripheral blood leukocytes, mononuclear cells or in unfractionated blood on a genome-wide scale. In particular I will argue that among available systems immunology approaches blood transcriptomics is very uniquely positioned in that it can add considerable breadth in addition to the depth that is inherent of systems approaches. By this I mean that while, as pointed out above, increased sophistication as well as "stacking" of technologies is ever increasing profiling depth, breadth – context – is at least as critical, if not more, for the interpretation of such vast datasets. Context is something that can be gained by capturing molecular profiles on a systems scale across a wide array of conditions (treatments, diseases, genetic and environmental factors, etc). Indeed, it is only when such a broad scope is attained that the perspective afforded by systems approaches becomes truly global. It is then for instance possible to determine if the activation of a given transcriptional program is specific to a disease or common to any number of diseases; the same can be said of a novel vaccine adjuvant, since data obtained from the profiling of approved vaccines or candidate adjuvants will allow the contextualization of the interpretation of a newly generated dataset. As will be discussed below it is also breadth and context that allows the identification of co-dependencies among the constituents of the system and the development of molecular repertoires (see also [6]). Also in my experience the answer to data overload is often simply... more data. Context can be purposely built into a study that will be designed to profile samples acquired across a vast range of conditions, or it can be gained a posteriori through the reuse of public domain data – given that precautions have been taken to ensure that the data is comparable. Indeed usable contextual data would be best obtained through coordination of efforts across research groups and disciplines, and more arguments going in that sense will be made below. In addition to depth and breadth a third complementary dimension, time, also contributes to potentiate systems profiling approaches especially when studies that are conducted involve research subjects. Indeed, adding this perspective accounts for the considerable inter-individual variability by measuring molecular as well as phenotypic fluctuations relative to a fixed point in time from the same individual. This is the last, but not the least, of the three dimensions that in my opinion can enable effective harnessing of systems scale profiling technologies but it will not be discussed in detail here.

So what characteristics make whole blood transcriptomics stand out in comparison to other systems profiling approaches as far as data contextualization is concerned? Blood is a tissue. Cellular heterogeneity is a limitation since changes in transcript abundance can be associated to either transcriptional regulation or relative changes in the abundance of different cell populations. Mining strategies such as in silico deconvolution that account for changes in cellular composition can mitigate this factor but it does not altogether remove this constraint on the interpretation of the data [7,8]. Nevertheless from the standpoint of the discovery and development of biomarker signatures only the performance of the test truly matters, and this is regardless of whether the change measured are due to transcriptional modulation or changes in cellular composition. It should also be noted that changes in cell composition that are detected via blood transcript profiling can be challenging to measure, even using methodologies such as flow cytometry. For instance neutrophil signatures do not correlate with neutrophil counts but with the presence of immature low-density neutrophil populations mobilized from the bone marrow [9]. We also observed via profiling of the blood transcriptome following vaccine administration a signature associated with an increase in abundance of antibody producing cells which are very fragile (especially to freezing) and require relatively sophisticated cellular profiling via flow cytometry for quantitation [10]. Indeed, accessibility and robustness of sample acquisition and processing methodologies makes up for what blood transcriptomics lacks in terms of granularity. Since blood is a homogeneous tissue no bias is introduced through sampling, which is in contrast with solid organs or tumors since cellular composition can vary considerably depending on the area where the sample is obtained. Evacuated blood collection tubes have been developed that contains a solution disrupting cells and precipitating RNA immediately upon homogenization by shaking [11,12]. The collection tubes can then be stored frozen at −20 °C indefinitely without further processing. The robustness of this sample collection method makes the approach amenable to collection on large scales and in particularly challenging settings. It also means that independent sample collections emanating from different studies could be consolidated for acquisition of transcript profiling data a posteriori. Notably, it is also possible to adapt the methodology for serial sampling using small volumes of blood, which adds a temporal perspective to the depth and breadth of blood transcriptome profiling studies. And thus it is the simplicity of this sampling and RNA profile stabilization method that makes whole blood transcriptome studies stand out among available systems approaches. Indeed, blood transcriptomics can be employed as a unifying theme or common denominator for bridging traditional research silos and provide in addition to depth contextual as well as temporal perspectives.

3. A global survey of the blood transcriptomics literature

I reviewed in 2010 the use of blood transcriptome profiling in immunology studies and the present article provides an update and fresh perspective on the current state of blood transcriptome investigations [2]. More recent reviews have focused on specific subject areas such as autoimmunity [13,14], Cancer [15], Stroke [16], the detection of erythropoietin gene doping [17], epilepsy prediction [18], and Alzheimer's disease [19].

Here a broader perspective was gained by conducting an extensive survey of the literature. This allowed for the mapping of the current landscape of blood transcriptome profiling studies. The evolution of this landscape over time was also examined. The first step consisted in building the following PubMed query to capture most relevant studies:

(blood OR PBMC OR PBMCs OR leukocyte OR leukocytes) AND ("systems approach" OR "systems immunology" OR transcriptome OR Transcriptomic OR "gene expression profiling" OR "transcriptional

profiling" OR microarray OR microarrays OR RNA-seq OR RNAseq OR "RNA seq" OR "RNA sequencing")

This query returned 19,947 articles; but only a fraction of these were actually associated with blood transcriptomic studies. Second, the search was restricted in order to identify studies that either pertained to diseases or conditions (e.g. sepsis, rheumatoid arthritis, autism) or species (e.g. rat, pig, dog). In order to accomplish this the operator "AND" was used followed by an ad hoc string (e.g. for sepsis: AND (sepsis OR septic OR septicemia OR septiceamia); for pigs: AND (pig OR pigs OR porcine OR swine OR hog OR hogs OR sow OR sows). In some cases where queries returned thousands of samples search for some of the terms were restricted to the title field (using the [ti] field operator). Next, the query results were curated manually to identify studies that employed blood transcriptome profiling. Review articles were excluded as were studies employing restricted panels of genes and studies focusing on microRNA profiling, which employ distinct assays to profile the "miRNAome" system in blood leukocytes as well as in serum samples. Since the unifying theme of this review is the transcriptome profiling of whole blood the studies that profiled abundance of RNA in isolated leukocyte populations were not recorded either. The final step, after the query results were curated, consisted in recording the cumulative study counts for each subject matter at 4-year intervals starting in 1998. Despite a genuine attempt at thoroughly surveying the literature some fields of investigation may have been overlooked or studies missed. Therefore the results presented here are if anything conservative estimates of the actual number of studies published that employed blood transcriptomics.

4. Blood transcriptome profiling studies in health and disease

Fig. 1 illustrates the evolution over time of the number of blood transcriptomic studies published across a wide range of subject matters. The studies here are entirely restricted to work carried out with human subjects and not animals. In total 505 unique studies were identified. Cumulative numbers of published articles are reported for 2002, 2006, 2010 and 2014. Consistent color-coding is used for different fields of investigations (e.g. darkest blue for infectious diseases, lime green for neurologic and psychiatric disorders). Labels indicate in any given year the subjects matters, ordered alphabetically along the *x*-axis, for which the number of publication reaches a count of 4, with the number of publications being plotted along the *y*-axis. Early adoption was observed in the field of autoimmunity, with Lupus, Rheumatoid Arthritis and Multiple Sclerosis reaching this threshold in 2006 [9,20,21], and with both cancer and hepatitis research field also contributing pioneering studies [22,23]. In 2010 study counts neared or surpassed 20 in all these research areas. This trend accelerated for Rheumatoid Arthritis and Multiple Sclerosis with counts nearing 40 in 2014, but on the other hand stagnated for Lupus with only 3 new studies added between 2010 and 2014, with a count of 23 studies to date. These differences can largely be attributed to the fact that investigations of Rheumatoid Arthritis and Multiple Sclerosis shifted from examining changes associated with disease processes to the investigation of mechanism of action of recently introduced biological drugs and especially the identification of markers predicting response to therapy [24–27]. No such treatment modalities are available to date for the treatment of patients with Lupus and the few recent studies have for the most part focused instead on patient stratification [28–30]. The number of blood transcriptomic studies investigating patients with hepatitis has also increased steadily over recent years, reaching a count of 29 to date, with studies reporting the characterization of interferon signatures in the blood of patients with hepatitis infection [31–33] and the identification of signatures associated with response to therapy [34,35]. But the largest increase has been in the number of publications utilizing blood transcriptome profiling in the context of cancer studies. Indeed, out of 57 studies to date 39 were published between 2010 and 2014 alone. This coincides with the mounting evidence over recent years of the efficacy of therapeutic strategies leveraging the immune system as a means to treat cancer, and more generally of the role played by the immune system in cancer pathogenesis [36]. Cancer studies have made use of blood transcriptomics for early disease detection [37–39] as well as the investigation of pathogenesis and therapeutic interventions [39–41]. Infectious diseases have also been extensively studied starting after 2006. In 2010 the number of sepsis studies reaches 18, starting from a count of only one in 2006. HIV, Tuberculosis, Influenza and Malaria blood transcriptomic studies have also become prevalent after 2010 [42–50]. Overall the number of studies for the 6 infectious disease subject matters surveyed reached 103 in 2014. It should be noted that space limited the number of infectious diseases or conditions that could fit on this figure. Indeed a significant number of studies pertaining to other infectious diseases that are not listed here have also been published, with for instance 11 articles to date on Dengue infection alone (e.g. [51–53]) or 5 on *Staphylococcus aureus* infection (e.g. [54,55]). Notably considerable interest has also emerged recently over the use of blood transcriptomic platforms for the profiling of in vivo responses to vaccines (e.g. [10,56–60]). Global changes in blood transcript abundance have also been investigated in patients with cardiovascular diseases, including heart diseases (e.g. atrial fibrillation [61] and heart failure [62]), hypertension (e.g. [63]) and stroke, with 21 studies published to date that have characterized blood transcript signatures associated with stroke and in some cases found to be predictive of recovery or complications (e.g. [64–66]). Interest has soared in recent years over the use of blood transcriptome profiling in studies involving patients with neurological disorders. In Fig. 2 a total of 53 studies have been cataloged in the Autism, Alzheimer or the broad "mental disorder" category, with 30 of which being published in the last two years alone. Studies range between investigation of Autism (e.g. [67]), to depression [68,69], anorexia [68], suicidality [70] or schizophrenia [71]. The considerable interest in use of this approach in the study of Alzheimer's disease has been largely spurred by the prospect of the development of a predictive blood transcriptomic diagnostic test [72–74]. Another field that has seen sustained interest and the prospect of the development of non-invasive clinical assays is transplantation medicine, with to date over 20 studies. These studies have investigated blood transcriptional signatures in subjects receiving liver, kidney, heart, lung or bone marrow transplants, with in this case the prospect of uncovering signatures that are predictive of operational tolerance or rejection (e.g. [75–80]). The effect of exposure to environmental factors, whether physical or chemical, on blood transcriptome signatures has also been reported in over 20 published studies (e.g. [81,82]), as have studies measuring in the blood changes in transcript levels associated with diabetes (e.g. [83–85]). Finally, investigative studies have also been published but in smaller numbers that pertain to various subjects matters ranging from respiratory diseases (16 studies, e.g. [86,87]), exercise (16 studies, e.g. [88,89]), trauma (9 studies, e.g. [90,91]), pregnancy (7 studies, e.g. [92–94]), allergy (6 studies, e.g. [95]). And this list could clearly be made much longer.

5. Blood transcriptome profiling studies in various animals species

Fig. 2 illustrates the evolution of the number of blood transcriptomic studies published for various animal species, including

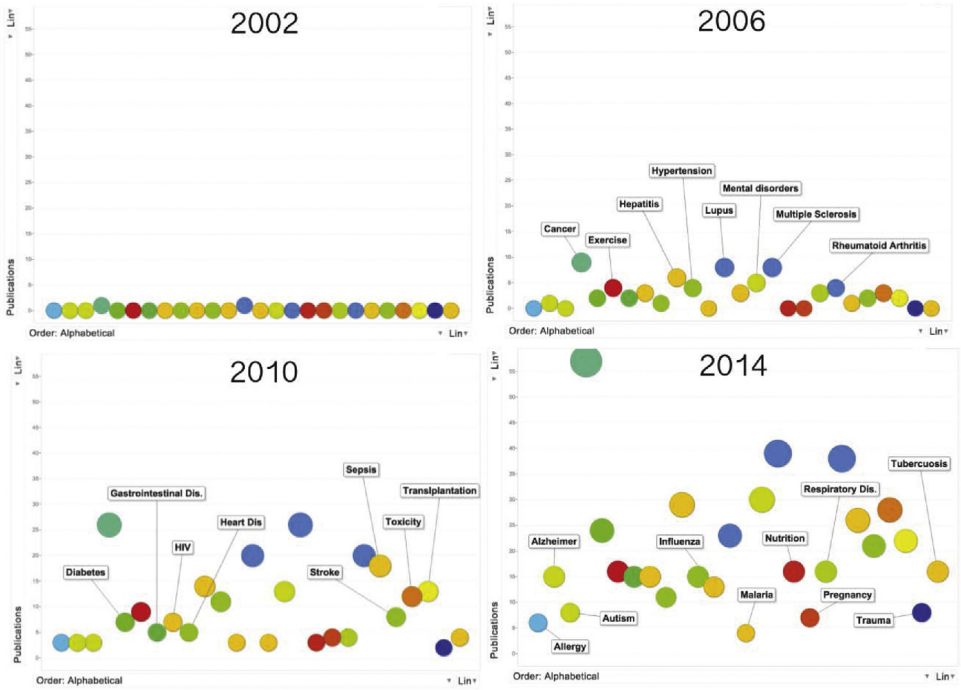

Fig. 1. Evolution over time of the number of blood transcriptomic studies published across biomedical research fields. The methodology used for retrieving and curating the literature is described in the text. Cumulative numbers of published articles are reported for different years. Labels indicate in any given year the subjects matters, ordered alphabetically along the *x*-axis. The number of publications is plotted along the *y*-axis.

species most commonly used in laboratory animal models, such as mice, rats and non-human primates (NHP), as well as economically relevant species such as the cow, pig, horse, sheep, goat, chicken, fish and others (e.g. dog, cat, dolphin). This survey revealed that systems scale blood transcriptional profiling has been adopted well beyond clinical and human immunology studies. As was the case of the human diseases surveyed above only a few studies were published in 2002 (top left panel). In 2006, a modest number of studies

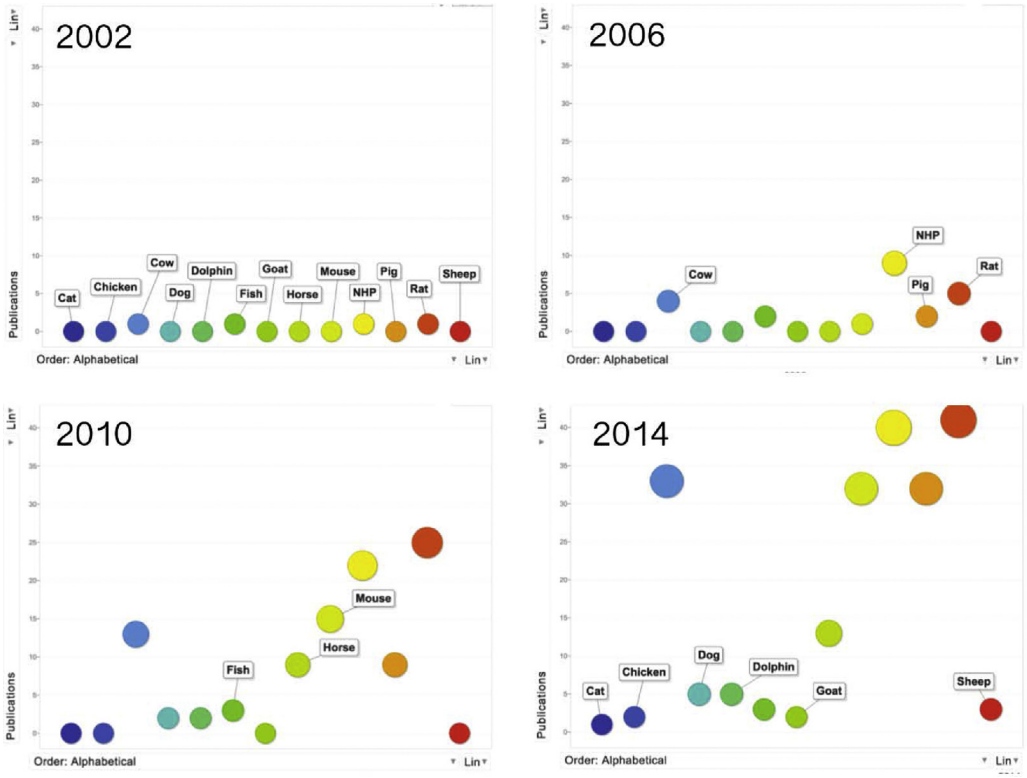

Fig. 2. Evolution over time of the number of blood transcriptomic studies published across different animal species. The methodology used for retrieving and curating the literature is described in the text. Cumulative numbers of published articles are reported for different years. Labels indicate in any given year the subjects matters, ordered alphabetically along the *x*-axis. The number of publications is plotted along the *y*-axis.

were published investigating the blood transcriptome of cattle and pigs and two model animals, the rat and NHPs. Interestingly, it is not until 2010 that a significant number of mouse studies are published, but this trend is sustained through 2014 and the number of published studies is now of 32, on par with the other "lead species", such as the rat (41 studies), NHPs (40 studies), cows and pigs (33 and 32 studies respectively). Mouse immunologists, who tend to favor organs such as the spleen, liver, lungs that are not readily accessible in human studies, do not commonly use blood. This fact may explain the relatively slow adoption rate observed here. Examining the number of publications by species in 2014 (bottom right panel) one can observe the gap that has formed between the "lead species" and the rest. A potential explanation may come from the limited availability of commercial microarray platforms for species other than Rat, NHPs, cows, pigs and mice. Unequal distribution of research funding is in all likelihood another contributing factor. And while availability of RNA-sequencing technologies disrupts the reliance on dedicated species-specific profiling array platforms, the cost and relative complexity of this approach means that it may take some time before the "lesser" animal species (on this graph) begin to close this gap. But some species have started to emerge, with most notably the horse (13 studies in 2014), and at present at least five studies that have been published investigating the blood transcriptome of dogs or dolphins. Interestingly some themes rapidly emerge when reviewing the blood transcriptomics literature for these different species. Indeed, rat studies are often designed to assess toxicity or pharmacological compounds or environmental factors (e.g. [96,97]), while studies in the pig or cow disproportionately revolve around resistance to infection, in particular *Mycobacterium bovis* in cattle and Salmonella in pigs (e.g. [98–100]). Reproductive biology studies are also well represented (e.g. [101–103]), which was in comparison a very minor theme in human studies. As can be expected these themes are common to most species of economical relevance. This is for instance the case of studies conducted in sheep and goats [104–106], as well as chicken [107] and fish [108].

Taken together this survey of the literature reveals the considerable breadth of blood transcriptomic studies, both across diseases and species. While this development is remarkable in itself it also offers distinct opportunities for advancing novel strategies for the exploitation of systems-scale profiling technologies. One opportunity lies with the use of the contextual data provided by those studies for the mining and interpretation of individual datasets as well as for the mining of large dataset collections. Having the ability to use blood transcriptomics as a unifying theme in order to bridge traditional research silos is in itself another significant opportunity, and would provide the means to exchange information and methodologies and to coordinate future large-scale blood transcriptome profiling efforts.

6. Meeting global health challenges

Blood transcriptomics has been widely employed in the study of immune processes involved in health maintenance and disease pathogenesis in human studies. It has contributed valuable insights, which in some cases have translated into novel therapeutic modalities [109], as well as diagnostic assays [72,110,111]. But successes have been few and far apart and blood transcriptomics studies have been slow to yield information that can be leveraged by physicians for clinical decision-making. Several factors may be contributing to this state of things. The current regulatory and intellectual protection environment is not favorable to the sustenance of business ventures, which have traditional been the means by which diagnostics tools are implemented and widely disseminated. Go or no go decisions come down to assessing cost vs. benefit. And at the moment there might just be too much risk to easily justify the upfront investment necessary for validating and productizing diagnostic assays, which results in poor prospects for translating promising results into clinical assays. But the cost and benefit balance might be shifted in low-income settings where disease burden is high and access to medical facilities is limited. It is especially true of course if benefit is assessed not only from monetary but also from a medical care standpoint.

Finding in cutting-edge molecular profiling technology the solution to medical challenges faced by destitute populations seems at first counterintuitive. The appeal of using systems approaches in such settings primary lies in the fact that systems profiling assays are by definition generic. Indeed, if everything that there is to be measured is in fact measured by such assays, then there is no need for arrays of dedicated kits or instruments for running different diagnostic tests. It theoretically would make it possible to run a comprehensive diagnostic lab with a single instrument running a single assay. Indeed, as illustrated in this review measuring molecular changes on a systems scale – in this case transcript abundance in blood – can be relevant to a wide range of diseases and conditions. It could potentially encompass infectious disease diagnosis – which are of especially high burden in developing countries – but also autoimmunity, cancer, neurological diseases, cardiovascular diseases, nutrient deficiencies, pregnancy, etc. Furthermore systems-scale profiling data have shown promise in enabling clinical decision-making not only with respects to differential diagnosis but also for the prediction of disease onset, or disease outcome, as well as assessing disease severity and monitoring treatment response [43].

But despite the very significant upsides described above, costs associated with running systems scale assays remains very significant. After all not only the instruments are expensive to purchase, but the assays are also costly to run – not to mention the infrastructure necessary to house such instruments and IT/bioinformatics overhead. Running such assays also requires extensive training. Furthermore, in many clinical applications, and especially for infectious diseases diagnostic, turnaround time is also critical, which would preclude adopting a highly centralized model. And even then, assay run time for systems -scale profiling assays tends to be calculated in a matter of days rather than hours. Therefore, where does the path forward lies for translating systems approaches into diagnostic tools in such settings? Technological breakthroughs are likely to bring the answer and shift this balance. This is illustrated by the recently introduced Oxford Nanopore's minION device, which enables high throughput sequencing on an instrument the size of a (large) thumb drive and that plugs directly into a computer's USB port [112]. The device is not reusable and costs about $1000 USD, furthermore suitability for clinical use in terms of robustness or reproducibility is unknown, but the advent of such a technology perfectly illustrates the fact that we may only be one or two technological breakthroughs removed from being able to implement systems approaches in settings where medical or laboratory infrastructure is quasi inexistent. Thus the prospect of leapfrogging the infrastructure-heavy western model for clinical diagnostic is very real, and could parallel the remarkable technology-enabled paradigm shift those countries have experienced, starting from no communication infrastructure at all to the ubiquitous use of cellular phones, without ever going through the intermediate step of using fixed phones connected to landlines.

If it is only a matter of time until such technology breakthrough(s) materialize what can be done in the meantime? We are in fact faced with a particularly tall order since technological advances will be necessary but not sufficient. Indeed, generating the data might be the least challenging task when compared with the extraction of reliable and actionable information from the vast amounts of data generated from a systems-scale profiling assay.

Fig. 3. The dream: Enable Clinical Decision-Making with a Universal Proximity Testing Device. Technological breakthrough will enable rapid and cost-effective proximity testing via portable devices capable of running large-scale profiling assays (1). The data thus generated will be transferred wirelessly to a mobile communication device such as a smartphone or tablet. In turn the data will be transmitted to a remote server via cellular or satellite networks where it will be processed and run against extensive reference databases of documented cases (2). Finally processed data will be communicated back to the mobile device and actionable information will be presented to health care staff to enable clinical decision-making (3).

It means moving beyond the proof of principle, which is what most studies published to date have offered, and in a coordinated effort build platforms for the development and validation of reference databases and algorithms that starting from large scale data will provide clinicians with information they can use for clinical decision-making for an increasing catalog of indications. Data can be generated on systems scales with technologies that are available now; granted that the practicality and price point is not as it should be to allow adoption, but the data is essentially the same as what those "next next-generation" platforms would produce. Numerous blood transcriptomics studies have already been conducted that are of immediate relevance to populations in developing countries [42,44,51]. Currently the prevalent model, as can be expected, consists in collecting blood samples at local sites in resource-limited countries to be run and the results analyzed in facilities in resource-rich ones. But here I would make the case that there is also an important need for building blood transcriptomic research capacity locally starting right now. I could come up with three main arguments in favor of this, with the need to: (1) Build local immunology research capabilities; firstly, there is, in my probably biased opinion, an obvious immunology research gap to fill. It is something I realized while visiting the Wellcome Trust-supported Oxford University collaboration within the Ministry of Health, Government of Laos, Mahosot Hospital Microbiology Laboratory in Vientiane, the capital of Laos. The facility is state-of-the-art according to western standards. But while the high infectious disease burden amply justify the clinical and research microbiology lab infrastructure, as is illustrated by this review much can also be learned from profiling host immune responses to pathogens, and the use of such platform can extend well beyond the field of infectious diseases. Other similarly equipped facilities exist around the world and are operated often through cooperation agreements between western organization and local authorities. The Pasteur institute's extensive international network is another such example. Building local immune profiling capacity also opens the prospects of building international surveillance programs, in order to monitor emergent infectious diseases and outbreaks. Indeed, host immune responses can be measured in the blood even when pathogens are not present and provide valuable information with regards to severity of disease and response to antimicrobial therapy and is therefore highly complementary to efforts focusing on direct pathogen detection. Having local data acquisition capacity in place becomes especially valuable in situations where outbreaks occur where pathogens are highly infectious and samples cannot easily travel outside of transmission areas, such as is the case with the 2014 Ebola epidemic. Here is my second argument: (2) Find solutions adapted to local unmet clinical needs; once capacity has been established for research it can be brought to bear to address local unmet clinical needs. Disease burden can vary considerably from one setting to another. Host and environmental factors are also likely to have an impact on blood transcriptional responses and biomarker development may require a "localized" – if not personalized – approach. These factors include for instance genetic background, disease burden (e.g. infestation with intestinal parasites), water quality or nutrition. It can be thus anticipated that databases containing reference profiles would be constituted by locally recruited cases. Weighting cost vs. benefits is here again likely to be the means by which the pace of translation of assay results in clinical decision-making will be determined and it is an assessment that will also need to be made locally on a case-by-case basis, based on the tools and resources available to medical personnel locally at any given point in time. Indeed, availability of adequate antibiotics supplies may for instance influence how a physician will treat information regarding the likelihood of a respiratory infection to be of bacterial or viral origin. (3) Prepare the ground for deployment of streamlined large-scale profiling technologies; thirdly, while the systems profiling platforms are not yet ready for prime time for this type of applications it is essential, taking technologies that are currently available to us, to model "the dream" as closely as possible, and to incorporate technological advances that will allow the miniaturization of instruments and lowering costs to the point where systems scale blood transcript profiling will be carried out using a drop of blood captured by a portable device that will generate data in a matter of minutes and relate the results to those available in a

central reference database through the use of wireless communication protocols (Fig. 3).

But how close are we really to becoming able to start building such research capacity in resource-constrained settings? In the short term the answer may lie in the use of streamlined "systems-like" approaches; or in other words targeted assays that are underpinned by systems scale profiling approaches. During the course of work carried out in various settings around the world (US, Spain, Macedonia, Thailand, Mexico, South Africa to name a few) our group generated blood transcriptome data from subjects with a wide range of diseases and conditions. From these and other datasets we have built modular repertoires – which consists in the identification of inter-dependencies amongst transcripts detected in human whole blood. This entirely data-driven approach consists of mapping relationships among group of genes on the basis of co-clustering patterns across a multiple datasets. This mining strategy has been described in detail in earlier publications and in a review article [6,113]. For instance we have most recently built a large gene co-clustering network using as input 15 different blood transcriptome datasets encompassing whole genome profiles of >1000 subjects and spanning infectious and autoimmune diseases as well as cancer. A total of 384 highly densely connected subnetworks of co-clustering genes, also called modules, were identified and can in turn serve as a stable framework for data analysis and interpretation of any blood transcriptome dataset. But the point I want to make here is that this modular repertoire framework can in turn be used to develop targeted assays; simply by selecting a few transcripts which expression patterns are representative of that of the entire module (tens to hundreds of genes) [6]. It should be noted that this selection process is entirely data driven and informed by systems-scale profiling. The end result is a targeted panel encompassing up to 270 transcripts (for 3 tiers) and as few as 90 transcripts (for 1 tier). Such a targeted "transcriptome fingerprinting assay" – can be run on meso-scale profiling platforms such as the Nanostring nCounter or Fluidigm Biomark systems. When run on a Fluidigm high throughput nanoliter-scale PCR system the reagent cost can be as low as $25/sample for one tier, and results can be turned around in a matter of hours. Sample processing workflow is straightforward as is the analytic pipeline, and web-based software applications are being implemented. This obviously falls well short of the dream, given the reliance on a relatively large and expensive instrument and the fact that while the selection of the gene panel has been informed by systems data the assay remains targeted–and thus can only be qualified as "systems-like". But the assay retains some important characteristics of systems approaches, given for instance that it is designed as a generic immune profiling assay that will detect perturbations associated with a wide range of immune mediated-diseases. It can thus be used for biomarker signature discovery, with the advantage of relying on PCR technology that is both sensitive and easily translatable in point of care diagnostic using conventional PCR technology. Because it does not measure everything this assay is less penalized by multiple testing corrections. This, combined with its higher sensitivity, can actually contribute to improve performances for biomarker signature discovery. This transcriptome fingerprinting-based strategy has been successfully benchmarked against a traditional microarray-based systems approach for the discovery of pre-symptomatic biomarkers with comparable performances obtained faster and at a much lower cost (manuscript in preparation). The transcriptome fingerprinting strategy and companion software superficially described here have been showcased in a recent innovation article calling for the democratization of systems immunology approaches [6]. Indeed the intent is to make this assay and software toolkits available as an open source platform investigators could add on and modify to fit their own needs and re-share for the benefit of the community.

Finally, it should also be noted that a very similar case could be made for the use of systems, or initially systems-like, approaches for veterinary medicine. As illustrated by the literature survey described above the translation potential of blood transcriptome profiling approaches has been demonstrated in animals of agricultural relevance. In fact, the same technology platform and translational research infrastructure deployed for the discovery of biomarker signatures of human diseases could be shared for that purpose. Notably integration of both human and veterinary medicine translational research activities aligns with the emerging "one health" or "dual purpose with dual benefits" concepts – to both human and animal health [114,115].

Obviously blood transcriptomics does not by any means tell the whole story. Other systems approaches will complete the picture by providing information about the state of other compartments such as the serum proteome and metabolome as well as the gut microbiome, which taken together will contribute to clinical decision-making. But profiling the blood transcriptome could be a good starting point. Its appeal lies in the fact that it is simple and that the level of maturity of the technologies it relies on is relatively high. Success breeds success, and getting started along that path would "only" take making a proof of principle for the use of a unifying systems or "systems-like" approaches in low resource settings to address unmet clinical needs across a wide range of diseases in humans and in economically relevant animal species.

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