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Retroviruses: a broad view of SARS-CoV-2 and its relatives, with a narrative essay on the current state of biomedical sciences

Alessandro Giuliani[1] **and Carlo Modonesi**[2]

[1]*Dipartimento Ambiente e Salute, Istituto Superiore di Sanità, Rome, Italy*
[2]*Dipartimento di Ricerca, Registro Tumori, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milan, Italy*

Abstract
The actual "pandemic" times, beside their burden of sorrow in terms of both victims, destruction of societal links and economic consequences, are an unprecedented occasion to give a closer look to the status of biomedical research. Beside the undoubted technological advances, the general impression is alarming: the fragmentation of science culture prevents any wise synthesis of the many aspects involved in a global phenomenon as SARS-CoV-2 epidemics. Here we try to acquire a "detached" view to some evolutionary and physiological aspects of the human-virus interaction highlighting the need to revitalize science by a strong departure from ultra-specialization toward a real integration of different fields of investigation.

Key words
- SARS-CoV-2
- retroviruses
- cancer
- ecoevolution
- exosomes
- integrative medicine

INTRODUCTION

The crisis triggered by the COVID-19 pandemic that has brought health systems to their knees is an alarming sign of the fragility of our way of living and thinking. It should not be thought that COVID-19 – just as the SARS and MERS epidemics that in 2003 and 2012 respectively kept the international health agencies in suspense – is an accidental phenomenon. Evidence of severe deterioration of environmental matrices and the collapse of ecological systems have been known for decades. Therefore, the pandemic crisis due to SARS-CoV-2 has deep roots and has to do with the crisis of an unsustainable development model that produces environmental damage, social inequalities and obscure ideologies, but still today receives support in most of the world. Within this framework, we cannot fail to turn our gaze towards the cultural stasis in which medical science navigates, as will be illustrated in more detail below.

In the 19th century and up to the 1970s, the natural sciences made remarkable progress in terms of discovering the fine structure of both living and non-living matter. These developments have had important effects on medicine, paving the way for so-called scientific medicine. These events made it very clear that medicine, as an applied discipline that absorbs the essential elements of its theories and practices from the natural sciences, became more and more dependent on new acquisitions coming from outside its sphere of influence [1].

The close relationship between medicine and natural sciences should today constitute the backbone of biomedical science and the heart of the philosophical thought that characterizes its normative principles. Unfortunately, the inter-disciplinarity that should solicit the current biomedical thought seems to have slowly dissolved. The new knowledge produced by the natural sciences has lost its power to feed the cognitive tools useful for exploring and understanding diseases [2]. This turning point in scientific medicine, which increasingly appears to be a privatized sector governed by financial interests, does not foreshadow good promises. Modern medicine increasingly makes use of advanced technologies for diagnosis and treatment (artificial intelligence, bioengineering, imaging devices, etc.), but at the same time progressively loses interest in protecting the health of citizens by promoting the primary prevention of diseases. The mere technological (and therefore instrumental) use of contributions from other sciences has made biomedical scientists to forget the scientific basis and tools they used in their day-to-day practice. Up until three decades ago, every neurologist knew the meaning of a Fourier analysis of an EEG and all experimental researchers had a clear understanding of the meaning of statistical significance, but today this is no longer the case [3]. Many influential members of the scientific community raised alarms regarding this cultural decay. One of the most acute and concise con-

Address for correspondence: Alessandro Giuliani, Dipartimento Ambiente e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: alessandro.giuliani@iss.it.

tributions, in our opinion, is that of Geman and Geman who compare the sense of wonder at the scientific progress perceived by a hypothetical scientist who travels in time between 1915 and 1965, with the much more modest excitement of another traveler moving between 1965 and 2005 [4].

The exaggerated emphasis on "technological-translational" aspects of medicine had a deleterious effect on the "tacit knowledge" dimensions of medicine by which physicians face the complexity carried by a patient in his/her wholeness [5]. This loss of complexity also highlights a dangerous detachment of modern medicine from its social dimension in an era, such as the current one, in which medicine increasingly needs to integrate conceptually and ethically with the natural and human sciences [6]. The thought and language of medicine have surrendered to the trivialization of its essential meanings and values, accepting the rules of media and breaking that important bond capable of reconciling tradition and innovation in an inseparable *corpus* of rules and knowledge. The time of the COVID-19 pandemic has led the general public to come into contact (very often for the first time and without much explanation) with a rather obscure jargon made up of "spike protein", "natural immunity", "RNA viruses", "epidemic curves" and many other technical terms. There is a general perception of a subtle threat that, in contrast to the main current threats to human health like cancer and cardiovascular accidents, directly affects habits as well as societal and inter-personal relations with an impact never experienced before. The fragmentation of knowledge in hyper-specialized fields has made it very difficult to grasp the essential issues of the various research areas that would allow a global synthesis understandable to public opinion. The lack of context awareness is at the basis of many forecasting errors impinging on the actual management of pandemics [7]. An "infodemics" made up of millions of papers dealing with SARS-CoV-2 has invaded all scientific and popular media from all fields of investigation. A recent paper by John Ioannidis [8] reported that mechanical engineers also had something to say about the SARS-CoV-2 highlighting a profound distortion of science's freedom of judgment by political and economic instances.

This puzzling situation prompted us to try a different approach completely detached from the day-to-day news: to look at SARS-CoV-2 from a broad perspective, taking into consideration some general ecological, evolutionary and cell biology implications raised by RNA viruses, with particular reference to retroviruses. Retroviruses are those viruses whose genetic material is a single-strand RNA molecule, which occasionally, after being "retro-transcribed" into the DNA of a cellular organism, can integrate into the host genome. Strictly speaking, SARS-CoV-2 is just an RNA virus and not a retrovirus. Its genetic material is not integrated into the host genome by reverse transcription; however, the true retroviruses whose genetic information is actually embedded in our DNA are the echoes of very ancient viral invasions in some respects not so different from SARS-CoV-2. Adopting a million-year perspective is, in our opinion, a potentially fruitful way to put in context the close integration among different aspects of the human-virus relations.

TWO SIDES OF THE SAME COIN

Evolution and ecology are two sides of the same coin. Evolution concerns biological change and genealogical relationships among organisms over time, while ecology is about the interaction networks among organisms and between organisms and the abiotic environment.

Although viruses lack a complete biological nature due to the absence of an autonomous metabolism and reproductive capacity, they exhibit evolutionary and ecological properties that determine much of their infectious behavior and the relationships they establish with host organisms. In the following, we will adopt a purely "operational" view focused on the human/viruses relations in both time and space, without entering into the debate of their living/non living character.

In the contemporary world, infectious diseases are a very important cause of suffering and death. Their incidence and geographical spread increased in recent decades, although scientists and politicians in the 1960s and 1970s believed that infectious diseases could be progressively neutralized thanks to economic and scientific progress (hygiene, better life conditions, medical advances, vaccination, technological development, etc.). This belief was formalized by the so-called "epidemiological transition theory" proposing that infectious diseases would decline in importance over time [9]. This was not the case, and since the mid-1980s the percentage contribution of infectious diseases to total mortality has increased even in developed countries and even excluding AIDS from estimates. Proponents of the epidemiological transition theory ignored the complex epidemiological patterns that characterize the waves of rise and fall of human diseases. Furthermore, they failed to clarify the disease profiles of other species, with particular reference to zoonotic diseases. In other words, they overlooked the ecology of diseases, especially the deep alterations that changes in land use, vegetation, climate, man-made environment, economy and technology cause in our relationships with pathogens (and possibly with vectors). These alterations can be appreciated, for instance, looking at the rapidly evolving resistance of pathogens to antibiotics and pesticides or considering the growing vulnerability of highly socially and economically stratified populations [10, 11]. The SARS-CoV-2 pandemic should remind us that our unbalanced interaction with the biosphere [12] raises many troubling challenges that healthcare systems around the world will face in the decades to come.

As we are realizing by examining the space-time evolution of SARS-CoV-2, any evolutionary process involving viruses and other infectious agents is the product of multifactorial dynamics and contingent events [13]. Health transitions are not linear and irreversible changes but complex processes involving possible re-emergence of diseases considered under the way of progressive reduction: many infectious diseases have an old cosmopolitan history of emergence, disappearance and recurrence [13]. The number of potentially infectious

contacts has exploded as global trade and travel bring goods, organisms and humans closer together than ever before. Nowadays, the longest intercontinental flight is shorter than the incubation period of any known infectious pathogen [13]. Meanwhile, the unexpected emergence and re-emergence of drug-resistant infectious diseases, the incidence of which is rapidly increasing, will change the global epidemiological scenario in the near future [14, 15].

Interestingly, RNA viruses, whose transmission cycles involve complex dynamics due to their evolutionary histories and their interaction with ecological factors, are the most frequent cause of emerging viral diseases [16]. A largely overlooked aspect of retroviruses is that they influenced the evolution of a large number of organisms, including our own species [17]. Evolutionary investigations suggest that retroviruses that infect vertebrates shared the biological history of their animal hosts for hundreds of millions of years [18]. In some ways, this is also consistent with the remarkable spread of retroviruses among modern vertebrates, which supports the hypothesis that their emergence dates back to around 450 million years ago. In other words, retroviruses could be contemporary infectious agents of the most ancestral animal lineages that appeared in the oceans of the Ordovician period [19]. We can safely say that viruses are an integral part of natural history – including, as we will see shortly, that of human beings – and therefore they do not represent only a "threat" of the natural world. The long evolutionary track we shared with retroviral sequences embedded in our genetic makeup had very important effects on physiological and pathological traits of our present lives [20].

RETROVIRUSES: A HISTORY OF SYMBIOSIS AND THE NEED TO RECONSIDER SOME FUNDAMENTAL PILLARS OF EVOLUTIONARY BIOLOGY

As first we need to go back from the very beginning, namely the definition of "what is life", this problem is with us at least from the time of Aristotle that basically defined a life being something that grows, maintains itself and reproduces, linking this definition to the concept of "purposed motion" or change [21]. After more than two thousand years and many heated philosophical debates, we are not so far from there, as the most popular definition of life stems from the presence of a metabolic activity (growing and maintaining itself as in Aristotle's definition) [22].

According to the above definition, a virus is not a living entity given it is neither capable of autonomous metabolism nor replication; on the other hand, viruses undergo mutation-based selective processes adapting their "phenotypes" to interaction with a host. Still more important, their relation with host presents the classical features to a parasitism-to-symbiosis transition often encountered in the natural world. This parasitism-to-symbiosis dynamic is particularly relevant in the case of retroviruses and contributed (together with other molecular biology evidences) to open a deep crisis of the still prevalent "modern synthesis" paradigm of biological evolution.

In a recent paper [23], Shapiro and Noble state:

"The common belief that the neo-Darwinian Modern Synthesis (MS) was buttressed by the discoveries of molecular biology is incorrect. On the contrary, those discoveries have undermined the MS".

In the paper they make a long list of last decades discoveries in molecular biology that undermine the basic pillars of the so called Modern Synthesis; they describe the impact on MS of these new discoveries by a metaphor borrowed by informatics.

"These 21st Century concepts treat the evolving genome as a highly formatted and integrated Read-Write (RW) database rather than a Read-Only Memory (ROM) collection of independent gene units that change by random copying errors".

In other words, organisms can change their genome in response to stress and the genotype-phenotype relationship is not only complex and far to be a one-to-one interaction, but can go the other way with phenotype that actively acts to modify genotype. The many experimental evidences of heritable phenotypic changes, what is most important in the case of retroviruses, is the falsification of the existence of an impenetrable Weismann Barrier separating somatic and germ line cells [23-27]. This means that viral genetic material integrated into somatic cells can be transmitted to the germline. Once the genome of cells that give rise to gametes (eggs and sperms) has been colonized by viral sequences, copies of the pro-viral DNA can be further amplified due to germline re-infection events [28]. These sequences are ubiquitous in vertebrates and in human genome accounting for around 8% of the genetic material (so largely outnumbering protein-coding genes) [29, 30]. For the most part, the sequences belong to the group of long-terminal repeats (LTRs) which also include the mammalian apparent LTR retro-transposons. Just like structural genes, ERVs (Endogenous Retro Viral sequences) undergo epigenetic regulation by histone methylation/demethylation and have a tissue specific expression level [30]. The term "endogenous retrovirus" does not refer to a biological entity distinct from other retroviruses, but simply describes any DNA of retroviral origin that has found its way into a host germline. This is probably the most intimate degree of symbiosis detectable in Nature: genes coming from retroviruses become part of the host genome at the same level of integration than the other genes. In this manner, the spread of ERVs may have accelerated the evolution of the host genome in largely unpredictable ways falsifying the necessity of the continuity of evolution through the slow accumulation of mutations and the consequent lack of any sharp distinction between micro- and macro-evolution, that is one of the main tenets of MS [23]. Phylogenetic analyses show that retroviruses cluster into five major groups with different host distributions, providing important insights into the classification and diversification of retroviruses [31]. Retroviruses underwent frequent host switches including many independent water-land transmissions, showing that the water-land interface is not a strict barrier for retrovirus transmission [31] and highlighting horizontal between species genetic transfer as an important factor in evolution.

The current debate on the natural/artificial origin of SARS-CoV-2 implicitly equates the consequences of an "artificially" engineered system to what normally happens in the natural world. The horizontal transfer of genetic information creates unexpected "shortcuts" between phylogenetically distant species that question the existence of a well-ordered "tree of life" in which evolutionary innovations (i.e. new species) emerge exclusively as a result of new ramifications.

ERVs are mainly regulatory sequences playing a crucial role in many biological processes like immunity, embryo development, tissue organization [32-34]. The way in which this role is exerted is very intriguing even from a pure "system science" perspective: ERVs exert a "digital control" that is more resistant to noise than analogic control. This digital control involves the so-called genetic "toggle-switches" (*Figure 1*).

The most common example of genetic toggle-switch is a bi-stable gene circuit consisting of two genes A and B which repress each other by imposing two different attractor states on the system of the two elements corresponding to: 1) A expressed at its typical level, B totally silenced; 2) A totally silenced, B expressed at its typical level [35].

Panel A reports two genes X1 and X2 represented as the poles of a feedback circuit: the edges represent the inhibitory action that one gene exerts on the other; these two inhibitory interactions have the same strength and are proportional to the concentration of the gene products. Panel B describes the dynamics of such a circuit in the X1/X2-concentration space. The point Sc, corresponding to an equal concentration of the two gene products, is a "saddle" i.e. a very unstable condition: if a small perturbation impinges on the system (e.g. slightly favouring A), the negative feedback exerted by A over B is greater than the one exerted by B on A. This initial asymmetry will grow up at each iteration until the system has only A-derived products. The opposite state (only B-derived products) occurs if we start with a little asymmetry favouring B.

Panel C adds to the X1/X2 space a third dimension called "quasi potential", a semi-quantitative estimation of the energy (and therefore of the instability) associated with each point in the X1/X2 concentration space. The same information is reported in the lower right panel which shows the presence of two minimum energy states favored by the two extremes of prevalence "A" and "B". This dynamic holds true at the single cell level and we can expect that for billions of cells in a culture or a tissue, a perfect overall balance of A and B products will be achieved due to the symmetrical character of the deviations.

Now let us look at the graph reported in *Figure 2* [36], in which the X and Y axes correspond to the cell expression profile of the same cell culture in two different instant of time. The vector points in the graph correspond to the expression levels of 23,000 genes; the evident linear arrangement of the graph is a natural consequence of the existence of a typical gene expression profile specific for cell type.

The scattering across the identity line is mainly due to Intrinsic (linked to the cell internal fluctuations in expression) and Extrinsic (caused by external noise) variability, some genes are in the so-called DE (Differentially Expressed) sub-space. They are single genes that by the effect of unknown stressors largely deviate from their ideal profile. The DE space is the preferred viewpoint for looking at possible phenotypic effects of drugs, diseases, genetic conditions. The DE space is continuous (i.e. analogical) because a gene can have a smaller or greater distance from the identity line. On the contrary, the long "whiskers" of *Figure 2* are made of On/Off "toggle" genes: in this case the variability is no more analogic but digital: a single toggle gene can be *off* (its expression is equal to zero) or *on* (and its expression level corresponds to its typical value). The puzzling point is that here we are not analyzing a single cell but populations of millions of cells in which we expect a

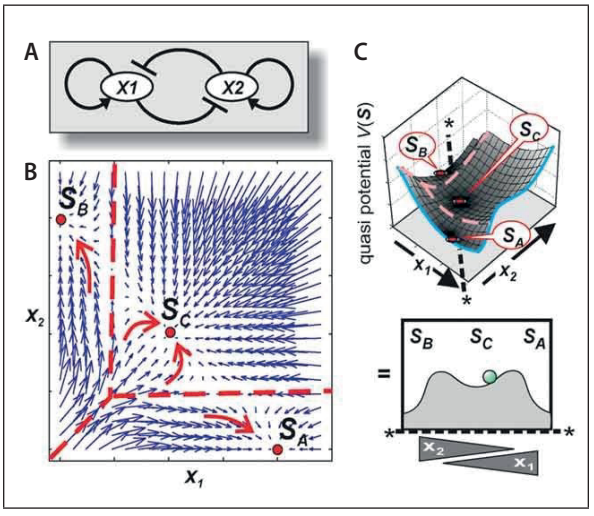

Figure 1
Toggle-switch behaviour (modified from Huang [35]),

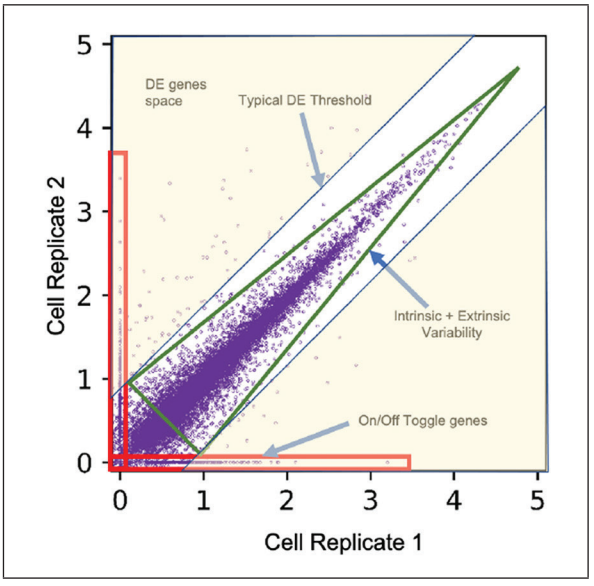

Figure 2
Whole genome expression space (from Giuliani *et al.* [36])

random distribution of the two alternative A and B solutions, with a consequent absence of the two "whiskers". The presence of hundreds of "single state" conditions tells us that the switches are tuned so to exert a digital yes/no control on the entire cell population. It is not by chance that the number of such "coordinated" toggle switches is extremely high in most critical conditions as embryo development and in general in multi-cellular organization. This digital control has a very ancient origin dating back to phages, viral particles infecting bacteria that switch between two discrete "lysogenic" and "lytic" stages corresponding to a host genome integrated and actively replicating behaviours of the viral genome [37].

The toggle-switch control, thus, appears as a main component of the dynamic regulation of gene expression, allowing for a more robust and accurate digital control with respect to continuous (analogic) variability. This kind of regulation is much more relevant in multicellular than unicellular systems pointing to a link between evolution of multicellularity and the need of a more reliable control to keep alive the physiological integrity of the tissues. It is worth noting the prevalence of ERVs in toggle switches, so highlighting the deep nature of virus-host symbiosis.

This as for the "sunny side": the above-sketched interactions describe the establishment of an unavoidable vital link between the expressions of genes due to the virus-host shared very long evolution track. On the other hand, the "dark side" concerns the involvement of ERVs in cancer (*Table 1*) and auto-immune diseases, that in turn are both "tissue-based" pathologies and in a sense can be considered as the price we pay for being complex and very finely integrated organisms [38]. Although the carcinogenesis mechanisms induced by ERVs have not yet been fully elucidated, the role of the viral sequences in the transformation of normal tissues into neoplastic tissues is widely recognized. Investigations of the past few decades suggest a broad association of different human ERVs with several cancers.

Extracellular Vesicles (EVs) are lipid bilayer-enclosed entities often containing proteins and nucleic acids. EVs resemble enveloped viruses in both structural and functional aspects. In full analogy with viral biogenesis, some of these vesicles are generated inside cells and, once released into the extracellular milieu, are called exosomes. Others bud from the plasma membrane and are generally referred to as micro-vesicles. The role of EVs as potent vehicles of intercellular communication stems from their ability to carry a wide range of biological macromolecules such as proteins, lipids, and nucleic acids. Regarding nucleic acids, DNA fragments, single and double-stranded DNAs, mitochondrial DNA and RNA species, such as mRNAs, miRNAs and a great variety of small non-coding RNAs have been detected in EVs [39]. Beside the still debated on common origin of retroviruses and exosomes [40], it is well established that a crucial factor in the control of infections is the accessibility of immune system cells to the foreign material. Exosomes – for their role in intercellular communication – play a key role in the dissemination of pathogens as well as host-derived molecules during infection either promoting or inhibiting host immunity [41]. The close interaction between exosomes and viral infections (including coronaviruses) is reviewed in Giannessi and colleagues [39]. In general, it is worth noting that exosomes are particularly rich in ERVs [38] and the demonstration of their transit from soma to germ line (so overcoming the Weismann barrier) [24] sheds light on the virus-host co-evolution.

All in all, a closer look at retrovirus-host interaction is telling us a very different and much more intriguing story than the one freezed in the central dogma of biol-

Table 1
Overview of the human ERVs detected in several cancers. The lack of X only means that there is no record of the human expression of that ERV for that cancer, and not necessarily that it is not present (from Vergara Bermejo *et al.* [28]).

	HERV-K	HERV-E	HERV-W	HERV-H	HERV-W	HERV-FRD	HERV-R	HERV-P
Breast	X		X	X	X		X	X
Lymphoma	X		X	X				
Leukaemia	X						X	
Endometrial	X	X	X			X	X	
Prostate	X							
Seminoma	X		X					
TCC			X					
Ovarian	X	X			X		X	
Melanoma	X							
Lung	X			X	X		X	X
Colon	X		X	X				X
Pancreas	X							
Sarcoma	X							
Urothelial/Renal	X	X	X	X	X		X	
HNSCC	X						X	

ogy and modern synthesis, endowed with many implications for human pathology [42].

CONCLUSIONS

Between the seventies and the nineties of the last century, some interesting essays were published on the fundamental definitions of health and disease, a subject of not so obvious interest within the scientific community but rather intriguing for sociologists, philosophers and historians of medicine [43-47]. Many authors argued that a true scientific discussion of health should start with the recognition of the relevance of complexity in human biology, medicine and psychology, clearly alluding to the systems theory of Ludwig von Bertalanffy [48]. The notion of health is closely connected to the notion of life: another thorny question that the biomedical community has historically avoided addressing, relegating it to philosophical reflection.

According to systems theory, distinct phenomena emerge at different hierarchical levels of biological complexity: atomic, molecular, cellular, individual, population (or social), ecosystem. Implicitly, the systemic perspective introduced the idea that biological and epidemiological exploration of the relationship between health and disease belong to the scientific realm of ecology, so that an exclusive focus on the molecular level as the ultimate causative organization layer does not allow to predict what happens at higher levels [48].

Today we know that the emergence of new detectable properties due to combination of many elements occurring at a given hierarchical level of complexity is fundamental to elucidating most of the biological dynamics. A very simple example of how reductionism is absolutely inadequate to explain the myriad of collective phenomena occurring in the natural world is offered by the so-called "herd immunity" (one of the many cases of obscure jargon mentioned in the first part of this contribution and abused by most mass media in relation to COVID-19). Indeed, herd immunity operates at the population level and clearly not at the individual level (according to the Edition 2020 of the Oxford English Dictionary, "Herd immunity is defined as resistance to the spread of a contagious disease within a population that results if a sufficiently high proportion of individuals are immune to the disease, as a result of vaccination against it or natural immunization").

In this paper, by taking retroviruses as case history (here we again stress that SARS-CoV-2 is not a retrovirus but only a RNA virus) we tried to give a glimpse to the intermingled status of biological knowledge. We tried to clarify how apparently heterogeneous issues like evolution, gene regulation, viral infections, ecology, and cell-biology are mutually consistent and ask for a global appreciation. For this and other reasons that can be coarsely defined as "attention to the context" we argue that an effort to seek new approaches is strongly needed in the health sciences and these new approaches must encompass the "serendipity" linked to the tacit knowledge of physicians [49].

Our goal was not to follow a blatantly "programmatic assertive" style of reasoning; on the contrary, in dealing with apparently very specialized problems such as RNA virus infections, we let the logical line be established by the need to simultaneously consider issues borrowed from a wide range of disciplines. The recent case of COVID-19 pandemics, at least in our opinion, made very evident the lack of a shared inter-disciplinary scientific culture and the urgent need to foster such a culture. The solutions that arise from strict reductionist approaches were in many cases unsuccessful and responsible for high costs for the health system [50]. We cannot forget that some alternative strategies have demonstrated a positive impact on the healthcare systems principally by implementing prevention and health promotion. Some other strategies have shown several advantages, like in the cases where different medical traditions are integrated to help patient engagement and compliance to self-care, reduced reliance on pharmacotherapy, and enhanced symptom control [51]. Particularly in the treatment of heart failure, the combination of traditional Chinese medicine with allopathic medicine has shown several benefits such as reduction of side effects and others [52]. Many biomedical scientists observed that these models have the potential to reduce the burden of both chronic and infectious diseases, lower the cost of healthcare, and offer a sustainable healthcare financial paradigm [53].

In conclusion, as aptly stressed by Georges Canguilhem [54], the complexity of human society and its current health and social needs require a systemic framework in which diseases are considered as the result of a negative interaction between multiple factors that characterize the human being as an individual and as a community. These approaches derive largely from a systemic view of human life and values, which highlights the fundamental principles of the organization of living beings from a perspective of well-being, equity and resilience.

Authors' contribution

The Authors contributed equally to this work.

Conflict of interest statement

No conflict of interest to declare.

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