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Current Pharmaceutical Design, 2015, *21*, 000-000

Beyond Numbers: The Informative Patterns of Immuno-Staphylococcal Dynamics

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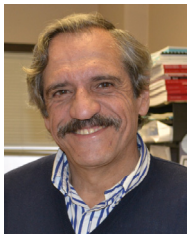

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Abstract: To evaluate new drugs, the immune system should be considered. Here we evaluated a proof-of-concept that uncovers bacterial-leukocyte interactions. Analyzing longitudinal leukocyte data from bovines infected with either methicillin-resistant (MRSA) or methicillin-susceptible (MSSA) *Staphylococcus aureus*, two methods were investigated: (i) an approach that assesses lymphocytes, monocytes, or neutrophils, separately, and (ii) a method that, using dimensionless indicators (products, ratios, or combinations derived from leukocyte data), explores the dynamics of leukocyte relationships in three-dimensional (3D) space and identifies data subsets of informative value.

The classic approach not always distinguished infected from non-infected cows. In contrast, the alternative approach differentiated non-infected from infected animals and distinguished early MRSA from early MSSA and late MRSA infections.

Discrimination was associated with the use of dimensionless indicators. When measured in 3D space, such indicators generated a very large number of combinations, which helped detect data subsets usually unobserved, such as non-overlapping infection-negative and -positive subsets, and several disease stages. The validity of such data subsets was determined with biologically interpretable data.

This graphic, pattern recognition-based information system included but did not depend on any one number or variable. Because it can detect functions (relationships that involve two or more elements), in real time, if shown reproducible, the analysis of complex host-microbial dynamics could be used to evaluate antimicrobials.

Keywords: Infection, MRSA, MSSA, complexity, systems, dynamics, three-dimensional, cutoff-free discrimination.

1. INTRODUCTION

Data analysis influences all research activities -including the evaluation of anti-bacterial drugs. For instance, to estimate the efficacy of antimicrobial drugs, data on the immune system are required [1].

Paradigms applied to analyze immunological data may influence pharmaceutical research. Here, such paradigms are reviewed, knowledge gaps and opportunities are identified, and an alternative method is described and evaluated.

The paradigm predominantly used to evaluate host-microbial interactions is reductionist [2]. While elegant and easily implemented, such paradigm results in: (i) errors, and/or (ii) information loss. Such weaknesses are associated with issues that involve data structure, biological functions, and data representation formats [3-6].

For instance, the approach used since 1947 ─also known as the '2 x 2 contingency table'─, does not measure time [7, 8]. Because early and late infections cannot be distinguished, the '2 x 2 table' paradigm does not inform on dynamics. Consequently, temporal concepts of pharmaceutical relevance, such as 'recovery from infection, after a treatment is implemented', cannot be easily distinguished with the classic approach.

Other problems relate to the central assumptions of classic statistics, such as data independence [9]. Because all elements of the immune system always interact with one another, all immune factors are inter-dependent [10].

To prevent these problems and to evaluate antimicrobials, *information*, not only *data*, is needed. Language illustrates the difference between such concepts. In language, the elemental components (letters) lack information. For instance, there is no meaning in the letters *o*, *m*, *e*, and *r*. Yet, when structured, information emerges: *more* is different from *Rome*. As complexity increases, so does information. To extract usable information, data structuring, not only data collection, is required.

To illustrate the relevance of relationship-related *data structuring*, let us consider a hypothetical situation that involves the coach of a soccer team —who needs to recruit 6 new players. It is not the same, for a soccer team, to recruit 6 players that play in the same position as recruiting 4 strikers and 2 goalkeepers. While the number of data points remains the same, the information retrieved by counts ('6 players') differs from the information provided by data structured as relationships (as in a '2: 1 strikers per goalkeeper' ratio). To generate information on biological functions, relationships involving two or more immune factors should be measured.

The need for data structuring derives from the highly *combinatorial* nature of immune-bacterial interactions [11]. Because such interactions vary over time, both data combinations and dynamics need to be measured. Because measuring dynamics is essential in drug evaluations, it follows that methods derived from static paradigms are not well fit.

To inform on biological functions and their dynamics, *data representation* also matters. The format chosen to analyze data may facilitate (or hide) critical information: it is not the same to read numbers from a table than visualizing the patterns shown by a three-dimensional (3D) plot —which, when movements (e.g., rotations) are analyzed, convey dynamic information [5, 12, 13].

These considerations support the need for paradigms that capture both dynamics and numerous data combinations. All biological systems (in particular, infectious diseases) are dynamic: over time, they change. What matters, in infectious diseases, is not to measure chronological units (seconds/hours/days), but to measure biological

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1381-6128/15 $58.00+.00

changes that follow some temporal sequence. That is, to generate robust or predictable systems, properties well conserved through evolutionary history should be considered ─properties applicable to any individual or population, even when such individual or population exhibits variability. Such properties include those described by chaos theory, i.e., those known as *non-linear dynamics*. When a system suspected to behave randomly reveals a distinct (non-random) structure, such system is dynamic [14]. Because dynamic systems are unpredictable, instead of conducting statistical analyses, uncovering their internal structure has been proposed [14].

To that end, here we analyze complexity [15]. Complex models may extract more information than classical approaches [16]. Based on pattern recognition, complexity may be easily interpreted, even without prior knowledge [15]. Three properties characterize complexity: (i) emergence, (ii) irreducibility, and (iii) unpredictability [17-19].

Emergence (also known as *novelty*) refers to the fact that complex systems are multi-level structures that reveal *new* features or functions only when the most complex (system-level) structure is assembled. *Irreducibility* means that *emergence* cannot be explained by or reduced to the properties of any one 'simple' (non-structured or low-level) variable. *Unpredictability* refers to the inability to predict emergence when only 'simple' and/or isolated variables are analyzed. For example, immunoglobulins express emergent properties, which are neither reducible to first principles nor predictable [20].

The properties of complex systems may be uncovered with dimensionless indicators [21, 22]. Dimensionless indicators are those that include ratios or products, alone or combined. They express relationships that involve two or more elements of a complex and dynamic system, such as the immune system. When measured in 3D space, dimensionless indicators can generate a very large number of patterns, uncovering information otherwise hidden.

Such indicators can be applied together with strategies that diminish errors ('noise') and/or increase discrimination -such as pattern recognition-oriented methods. For instance, primary data (the data actually collected in a study) can be structured to reveal some desirable properties, such as a single (one data-point wide) line of observations. Such structure diminishes information loss while enhances discrimination because, when temporal data are assessed within a single line, data movements, over time, can only take place along (not across) such line. Such feature is directly interpretable because, when infectious disease data are investigated, one end of such line represents 'health' and the opposite end reflects 'disease', i.e., any temporal data movement, no matter how small, is informative [23, 24].

Here we evaluated a proof-of-concept that explored 3D, complex patterns of infectious disease data. Due to the medical relevance associated with methicillin-resistant *Staphylococcus aureus* (MRSA) [25], data collected from a MRSA-infected cow, and from cows infected with methicillin-susceptible (MSSA) staphylococci were investigated. Three questions were asked*:* (i) did *S. aureus*-related infections reveal properties typical of complex systems? (ii) did such infections reveal other properties that may influence diagnosis and/or prognosis? and (iii) if complex properties were demonstrated, could such properties distinguish dynamics and/or help improve diagnosis and prognosis of staphylococcal infections?

2. MATERIALS AND METHODS

Materials

Leukocytes were collected from MRSA- or MSSA-infected cows. The original data were reported before [26, 27]. Fifty-two (24 MSSA and 28 MRSA) observations were investigated. In the MSSA study, six lactating cows were inoculated intra-mammarily with a MSSA isolate and milk samples were tested before and up to 14 days after challenge [27]. In the MRSA study, all mammary quarters of one spontaneously infected cow were investigated at days 1-5, 8, and 9 (7 tests), where day 1 was the first day MRSA was isolated in milk [27]. Milk leukocyte data on neutrophils (N), monocytes (M) and lymphocytes (L) were collected and structured as described earlier [26].

Method

Complexity was investigated with a two-step process meant to (i) detect and differentiate distinct data subsets, and (ii) identify, with biologically interpretable data, the meaning of distinct data subsets. In the first step, several hundreds of data combinations were randomly generated and assessed with bi- and three-dimensional (2D, 3D) plots. Each data combination included two or more dimensionless indicators (DI). Any set of two (or more) DIs included all the data of all three (lymphocyte, neutrophil, and monocyte) cell types. A DI was any construct that included products and/or ratios (alone or combined) derived from leukocyte data. For instance, the product resulting from multiplying the monocyte percent by the neutrophil percent (the M% * N%) is a dimensionless indicator. Because the number of data combinations created in the first step was very large, identifiers lacking any meaning (expressed with italics, e.g., *A, AB, AAB*) were assigned to dimensionless indicators.

DIs were hypothetical measures of relationships which, when explored in 2D or 3D space, could reveal distinct (non-random) patterns, such as perpendicular data inflections. Such patterns were used to detect and distinguish data subsets. A distinct data subset was defined as a group of observations that did not overlap with other observations or subsets. Data subsets were identified by 'redundancy': at least two different sets of dimensionless indicators were utilized to identify each data subset.

The goal of the second step was to determine the actual (biological) content of each data subset identified in the first step. The second step did not consider DIs (temporary tools only used in the first step) and did not look at all the data: it analyzed each data subset, separately.

In the longitudinal-experimental (MSSA) study, molecular data (CD11b and CD3) were collected and assessed by flow cytometry as described elsewhere [26]. These molecules were measured because they mediate the (lymphocyte-mediated) antigen recognition and the (phagocyte-related) cell activation processes (CD3 and CD11b, respectively). The data were expressed as mean fluorescence intensity (MFI or receptor density per cell) and also as percent of cells expressing such markers. Molecular indicators were used unadjusted (e.g., the CD3 MFI) or adjusted (e.g., the CD3 MFI/CD3+ lymphocyte percent, or CD3 receptor density per CD3+ cell). They could also be adjusted in reference to a relationship, such as the N/L ratio, which increases in early inflammation and can be expressed with a novel index ([CD3 MFI/CD3+ lymphocyte %] / [N/L]). Other adjustments utilized the monocyte/neutrophil (M/N) and/or the mononuclear cell/neutrophil (MC/N) ratios which, if increased, denote the resolution phase of the immune response [28].

Considering both cellular and, when available, sub-cellular (molecular) cell marker-related information, infections were regarded to be either 'early' or 'late.' Such terms are relative and refer to the temporal sequence of events observed within an inflammatory response (that is, they do not express chronological units, such as hours or days). MSSA or MRSA can invade mammary parenchyma and be shed in the milk intermittently over time. For this reason, a chronic infection may be regarded as a recurrent one. Because 'early' leukocyte-microbial interactions occur before 'late' ones, animals showing recurrent infectious episodes show 'early' responses in every episode, and animals infected only once also display a 'late' inflammatory stage. Such data structures were used to measure relationships taking place within or across biological scales. For instance, the [CD3 MFI/CD3+] / [N/L] index meas-

ured: (i) a molecular (CD3 MFI), (ii) a cellular (the percent of CD3+ lymphocytes), (iii) a supra-cellular (the N/L ratio) scale, and (iv) the overall interaction.

This approach also evaluated different levels of complexity. For instance, the N/L ratio estimated a low level of complexity, while the product of M/L times L+M/N, divided by the product of MC/N times M * L measured a highly complex relationship.

In brief, validation of this approach was conducted with a two-step process: (i) first (and using a proprietary algorithm), hundreds of dimensionless indicators were created, which identified distinct data subsets, and (ii) later, the bacteriological and leukocyte data associated with each pattern were retrieved and analyzed. This approach did not establish any cut-off or limit on the number (or complexity) of any one element or step. By measuring many interactions, scales, and levels of complexity, this approach was expected to uncover patterns otherwise missed. While unknown before data collection, data patterns were revealed at the end of this process.

Classic Analysis

Both statistical tests (analysis of medians, Mann-Whitney test) and uni-, bi-, and three-dimensional plots were conducted with *Minitab 16*, Minitab Inc., State College, PA, USA.

3. RESULTS

Both 'simple' (non-structured) indicators and those that possessed a low level of complexity (a single ratio or product between two variables) failed to distinguish *S. aureus*-negative from –positive observations. Regardless of the (molecular, cellular, or supra-cellular) scale analyzed, a substantial number of overlapping data points were observed (blue boxes, Figs. **1A-C**). In contrast, when three-dimensional (3D) plots were utilized and time was considered, all 0 day[s] post-infection (DPI) observations (data collected before cows were infected) were distinguished from all 1 DPI data points (Fig. **1D**). However, some of the later (4-10 DPI) observations overlapped with either 0 or 1 DPI observations (Fig. **1D**). Such data representation also showed a circular data structure (arrows, Fig. **1D**). While the assessment of dynamics was more informative than alternatives that did not measure interactions or time, the 3D approach did not differentiate all biological conditions and, in addition, it displayed circularity - a structure that lacks zero values and, consequently, poses diagnostic problems.

To both capture multi-scale interactions and diminish data variability (associated with non-standardized metrics), molecular data (CD11b MFI, CD3 MFI) were measured without and with adjustments. The dynamics of molecular data were adjusted to the N/L ratio (a relationship known to increase in early inflammation). While both CD11b and CD3 increased between 0 and 1 DPI and so did the N/L ratio (Figs. **2A**, **B**, respectively), the adjusted CD11b/ [N/L] index decreased at that time (red boxplots, Fig. **2A**). Similarly, the adjusted CD3/ [N/L] index diminished between 0 and 1 DPI (sky blue boxplots, Fig. **2B**). That was so because the magnitude of the N/L increase (the denominator of the composite index) was larger than those of the molecular indicators (the numerators). Therefore, findings supported two conclusions: (i) the dynamics of complex systems (such as those of the CD11b MFI /[N/L] system) can differ from those of its components; and (ii) the variability likely to occur when different biological scales are measured can be prevented when composite indicators are applied.

When multi-scale adjusted indicators were utilized together with dimensionless indicators and temporal data movements were depicted with qualitative indicators (arrows, Figs. **3 A-F**), the problem shown in Fig. **1D** was ameliorated. For instance, when indicators that captured complex relationships (identified as *BAG*, *AAE*, and *X*) were plotted together with either CD11b MFI/[N/L]

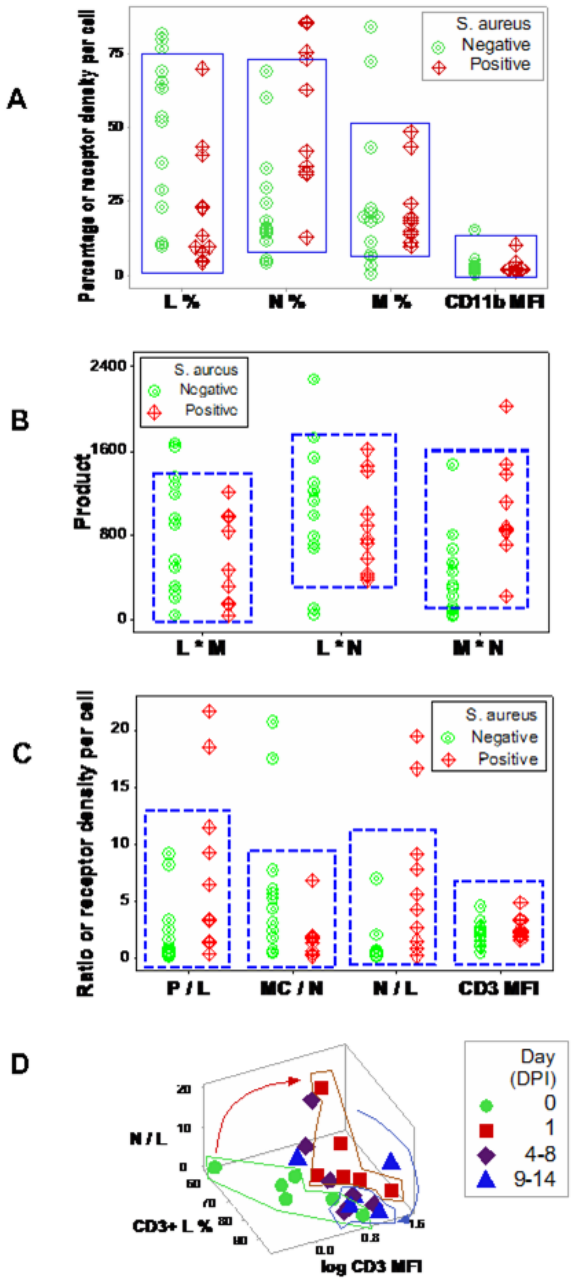

Fig. (1). Properties of infectious disease data: problems and opportunities. No discrimination was possible when simple (non-structured) indicators were analyzed, such as the percentages of lymphocytes (L), neutrophils (N), or monocytes (M, **A**; , or low-complexity indicators were utilized, such as products (**B**) and ratios (**C**). Molecular data (receptor density per cell, expressed as median fluorescence intensity or MFI, e.g., CD11b receptor density per monocyte) also failed to distinguish non-infected from infected individuals, (**A, B**). Blue boxes indicate the range of culture-negative and -positive data overlapping (**A-C**). When time was explored and a three-dimensional plot was used, an oscillatory or circular process was observed, which discriminated, without data overlapping, all 0 day post-infection (DPI) from all 1 DPI observations (**D**). Because some of the late (9-14 DPI) observations overlapped with either 0 or 1 DPI data points, the simple use of a 3D plot, while more informative than the alternative, was not adequate to distinguish all biological conditions and, in addition, revealed *data circularity* --a feature that renders classic metrics (e.g., means and confidence intervals) non-operational, because circular data lack true zeros and endpoints (**D**).

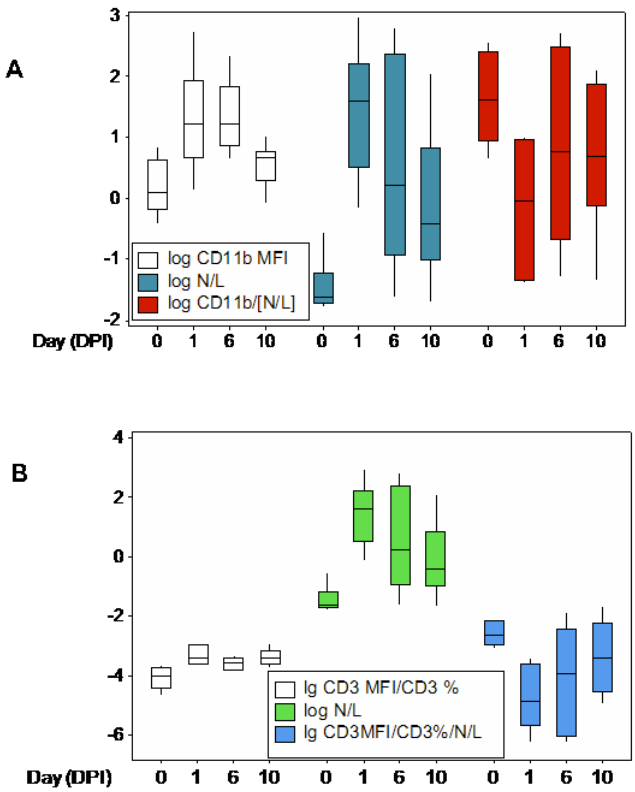

Fig. (2). Multi-scale relationships --differentiating the dynamics of the system from those of its components. The relevance of exploring complex, multi-scale systems (as opposed to measuring simple and single-scale components) was demonstrated when adjusted indicators were investigated. While both the CD11b MFI and the N/L ratio increased between 0 and 1 DPI observations, the adjusted CD11b MFI/ [N/L] indicator decreased at that time period (red boxplots, **A**). Similarly, the CD3 MFI per CD3+ lymphocyte increased between 0 and 1 DPI, while the adjusted indicator diminished (sky blue boxplots, **B**), revealing that the dynamics of the system may differ from those of its components.

(identified as 'Cd11M / [N/L]' for brevity) or [CD3 MFI/CD3%] / [CD11b MFI]/[N/L] (identified as '[Cd3M/Cd3] / [Cd11M/[N/L]' for brevity), perpendicular data inflections were observed, which denoted changes biologically interpretable (here described as 'recovery', when they reflected changes that approached disease-negative data subsets, or 'chronicity', otherwise, Figs. **3A-D**). Early and late inflections were distinguished when 3D plots were utilized (Fig. **3G**).

Applications of this approach were explored. A perpendicular data inflection distinguished MRSA from MSSA data points (Figs. **4 A**, **B**). To compare only observations known to be associated with infections, 0 DPI MSSA observations were removed. The patterns of infection-positive only data did not differ from those that included infection-positive and -negative observations (Figs. **4 C**, **D**). While MRSA temporal data were not comparable to the MSSA temporal data ('day 1', in the MRSA dataset, only indicated the first day MRSA was identified, not necessarily the first day of infection), the group of MRSA data points shown as a vertical subset could be tentatively labeled as 'early' observations (Fig. **4D**). Such temporal classifier was justified by the fact that, in the MRSA subset, data points recorded 7-9 days later were not included in the vertical ('early') subset. Thus, both MSSA and MRSA infections revealed a similar temporal pattern (arrow, Fig. **4D**). Additional data structures also showed orthogonal data inflections (Figs. **4E-H**). Because data points were mainly observed along plot edges and, in addition, a single line of observations (with inflections) was generated, data subsets were distinguished and interpreted, regardless of any numerical value: arrows reflected temporal data directionality (i.e., disease progression, Figs. **4 F**, **H**).

To validate this approach, biologically explicit data (leukocyte percentages of MRSA and MSSA infected cows) were explored focusing on the subsets previously identified ('early MRSA' and 'other observations', Fig. **4D**). The 'early MRSA' descriptor was justified: it displayed responses typical of early inflammations, e.g., lower L% than in later responses (P<0.01, Fig. **5A**). In spite of statistical significance, overlapping data distributions prevented from fully discriminating such categories (Fig. **5A**). While indicators that assessed a low level of complexity (simple products or ratios) also reached statistical significance, non-overlapping data subsets were only observed when an index that included a product and a ratio (the [M * N] / L), i.e., an indicator of higher complexity), was utilized (broken line, Fig. **5B**). Yet, such combination failed to distinguish MRSA from MSSA observations (Fig. **5C**). MRSA and MSSA patterns were differentiated when, in addition to data combinations that included ratios and products, 3D relationships were considered (Fig. **5D**).

4. DISCUSSION

It has been stated that the major challenge faced by Systems approaches is 'the experimental hurdle.' That refers to investigating, simultaneously, phenomena that may occur across levels, which result in multilevel interactions [11, 29]. That problem was here addressed with a complexity-oriented approach that assessed dynamics and uncovered hidden interactions.

S. aureus-related infections revealed properties typical of complex systems: *emergence*, *irreducibility*, and *unpredictability* were documented. The patterns observed were not predicted by or reduced to the properties of any one simple indicator, even when indicators of low complexity (such as the N/L ratio or the M * N product) were considered. Instead, patterns of informative value were associated with dimensionless indicators of higher complexity -and only when analyzed in 3D space. New or emergent patterns distinguished not only *S. aureus*-negative from -positive observations, but also early from late infections. In addition, MRSA and MSSA infections were differentiated.

Properties that, potentially, could lead to non-interpretable data, were also documented (such as *circularity*). When the data structure is circular, because there is no true zero and no endpoint, classic statistical tests cannot be applied [30, 31]. While the data circularity-related problem has been addressed in other fields (such as navigation-related sciences) with statistical tools that apply to the analysis of surfaces, here such approaches were not considered because infectious disease data displayed patterns that involved not only surfaces but also volumes (3D relationships).

An additional property that may result in diagnostic problems is the 'cost of dichotomization' [32]. That term refers to both false-negatives and false-positives, which invariably emerge when a numerical cut-off is imposed on continuous data (such as leukocyte data) and observations above or below such cut-off are then assigned a discontinuous (discrete) label, such as infection-negative and infection-positive. Had any numerical cut-off been applied to the continuous data shown in Figs. **1 A-C**, many false-negatives and false–positives would have been generated. Such problem was prevented when a pattern recognition-based approach was used, in which distinct (graphic) features, such as perpendicular data inflections, differentiated data subsets, as shown in Figs. **3-5**. Such approach was facilitated by both the use of a single line of observations and arrows that informed on temporal data directionality.

Because *temporal data directionality* generates inferences [33], qualitative tools, such as arrows that denote the direction followed by data movements, help determine whether any one data point

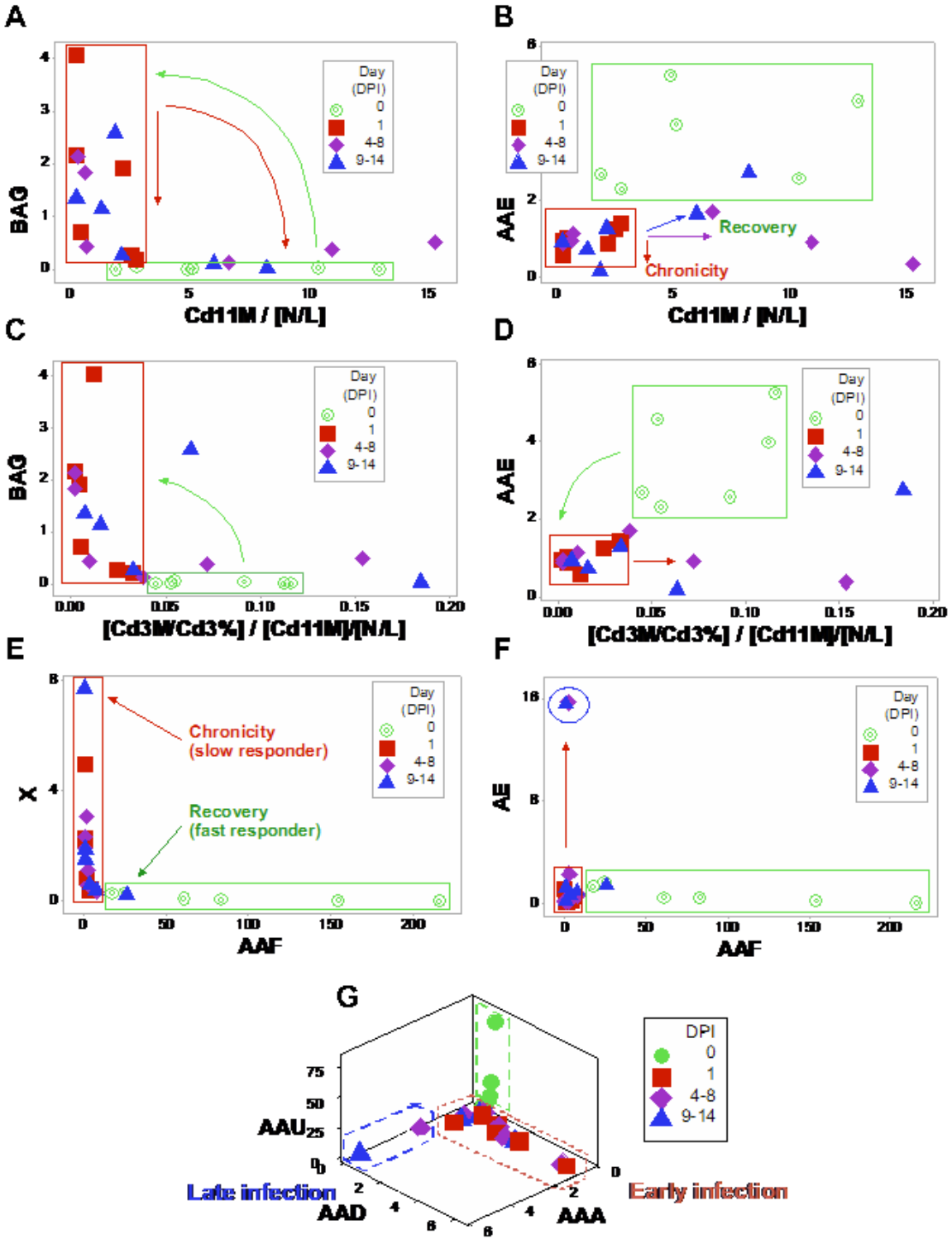

Fig. (3). Single line- and dimensionless indicator-based discrimination. When dimensionless indicators (e.g., *BAG, AAE*) were explored together with adjusted indicators, temporal data directionality provided interpretable information, regardless of any numerical value (**A-D**). Temporal data movements (arrows, **A-D**) indicated progression toward the disease-negative (0 DPI) or -positive (1 DPI) data cluster. Four data structures differentiated 0 from 1 DPI observations without data overlapping (**A-D**). When individual observations (not patterns) were analyzed, slow or fast immune responders were distinguished. For instance, two observations (corresponding to two different animals), collected between 9 and 14 DPI, were either located within the vertical subset (indicated by an red arrow, **E**) or the horizontal subset (indicated by a green arrow, **E**). Consequently, one animal could be regarded as a fast responder (the observation included within the horizontal subset, i.e., an animal already recovered), while the other observation could be viewed to represent a slow responder. The use of dimensionless indicators was associated with a single (one data point-wide) line of observations (**C, E, F**). These features were also observed in 3D space, when prechallenge, early, and late infections were distinguished by a triple perpendicular data inflection (**G**).

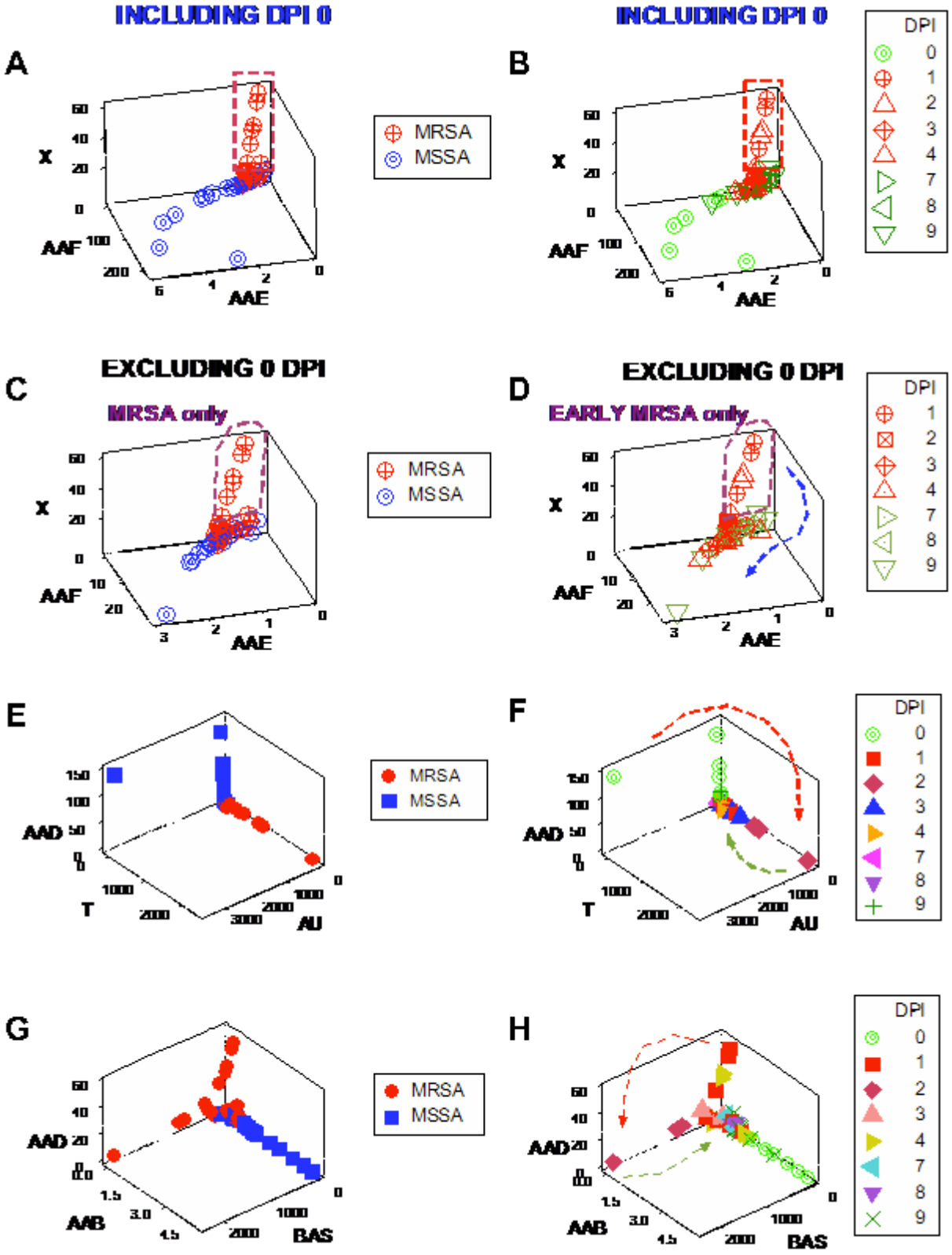

Fig. (4). Dimensionless indicator-based data subsets -MRSA and MSSA patterns. A perpendicular data inflection distinguished early MRSA observations from other data points (box, **A, B**). A similar pattern was observed when 0 DPI MSSA data points were eliminated (**C, D**). Temporal data indicated the vertical subset was only composed of early (1-4 DPI) MRSA observations (**D**). Such patterns were confirmed through redundancy: other data combinations of dimensionless indicators revealed similar information (**E-H**). Perpendicular data inflections distinguished MRSA from MSSA infections (**E, G**). When time was considered, at least two perpendicular infections were observed over time (**F, H**). Note: because MRSA data were non-experimental, 'day 1'MRSA' data refer to the first day inflammation was observed (not necessarily the first day after inflammation developed).

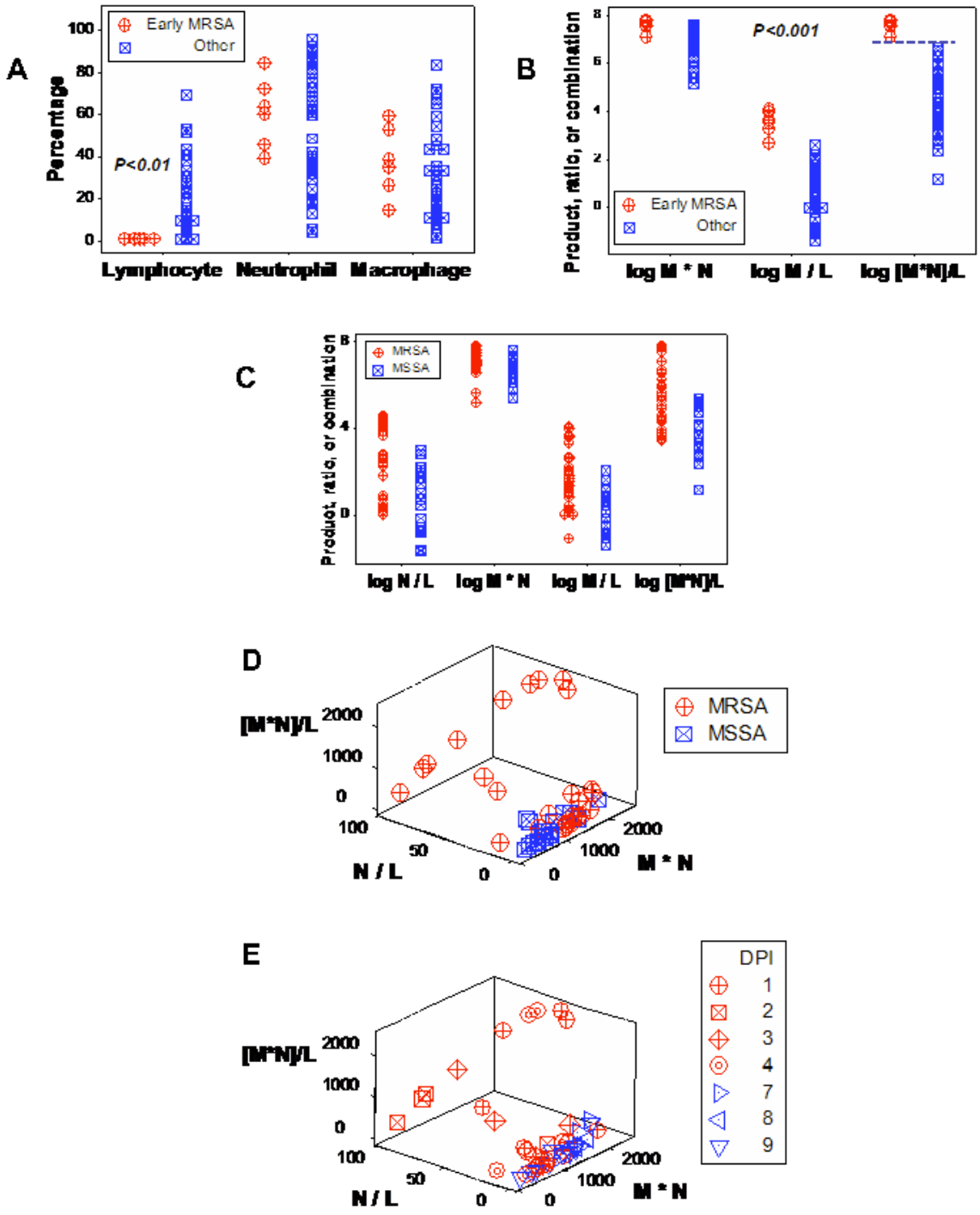

Fig. (5). Validation of the complexity-oriented method --MRSA and MSSA patterns. When 0 DPI MSSA values were not analyzed and percentages were considered, the early MRSA subset displayed a statistically significantly lower median L% than the remaining observations (P<0.001, Mann-Whitney test, **A**). However, no leukocyte percentage fully distinguished 'early MRSA' from 'other observations': a substantial data overlapping was observed. When products or ratios were analyzed, alone or combined, all analyses reached statistical significance (**P**<0.001, Mann-Whitney test, **B**). However, only the combined indicator (the [M*N[/L) discriminated without any data overlapping (broken line, **B**). Yet, neither the combined ([M * N] / L) indicator nor its components differentiated MRSA from MSSA infections (**C**). However, when the same indicators were assessed in 3D space, a cluster of data points composed only of MRSA observations was observed (**D**). Such cluster corresponded to the earliest (1-3 DPI) MRSA observations (**E**).

represents an infection or not as shown in Figs. **3E**, **3G**, and **5D**. When at least two temporal data points are simultaneously measured (and they are connected with an arrow), the arrow will indicate whether the most recent observation is moving toward the infection-positive or -negative pole of the data distribution, as Fig. **4E** illustrates.

The practical value of data directionality depends on the structure of the construct. When indicators are designed to produce a single (one data point-wide) line of observations, data variability is eliminated from all dimensions except one: that of the single line of observations. Single lines of observations not only diminish noise but also facilitate cutoff-free, confidence interval-free information. Such structure prevents errors associated with (cut-off mediated) false-negatives and false-positives [32].

Data subsets were validated with biologically interpretable data. For instance, the two subsets identified in Fig. **4D** (early MRSA and other observations) showed non-overlapping data distributions when the [M * N] / L composite indicator was investigated (Fig. **5B**). Such differentiation was not possible when the data were analyzed without structuring (Fig. **5A**).

Findings revealed applications of potential value in pharmacology. In evaluation of pharmaceutical drugs, major issues of practical relevance include: (i) to detect data subsets, without any data overlapping; and (ii) when a new drug is investigated, to distinguish whether results are due to the drug or the individuals tested in the trial (e.g., slow or fast immune responders). Supporting such needs, four data structures discriminated, without overlapping, 0 DPI data points from other observations (Figs. **3A-D**), and (ii) slow and fast responders were differentiated (Fig. **3E**). Such feature could evaluate antimicrobial drugs: temporal data movements along such line could indicate drug efficacy (as shown by arrows, Figs. **4F**, **H**).

Such applications could be conducted in real time. Because some complex data structures were closely correlated with molecular-based indicators, complexity can be detected using complete blood count data, i.e., avoiding a time-consuming procedure, such as flow cytometry.

Interpretable and usable information seemed to be the result of the level of complexity built into the data structure. Supporting the view that more or new information can be extracted when higher levels of complexity are investigated, the macrophage/lymphocyte ratio differed between early MRSA+ bovines and other observations -a relationship detected without any overlapping when a composite index (the [M* N] / L) was utilized (as illustrated in Fig. **5B**). While no previous study has explored the role of the M:L interaction in staphylococcal infections, this proof-of-concept supports the view that such interaction may be considered in evaluation of antimicrobials.

Because some complex data structures revealed a single line of observations, data movements along such line could indicate whether new drugs are efficacious or not, even before laboratory tests are performed. Because the same single line of observations can be applied to evaluate infection in any species, this approach facilitates comparisons among studies conducted with different bacterial strains or antimicrobials. Because this proof-of-concept can be implemented in real time, if confirmed, it may provide cut-off-free, confidence interval-free, earlier information on multi-scale, multi-dimensional dynamics of host-pathogen interactions.

CONFLICT OF INTEREST

No financial contribution was received.

ACKNOWLEDGEMENTS

ALR and RP generated the data. ALH performed the analysis. All authors participated in the design and reporting of the study. The authors thank the comments received from participants of the conference on infectious diseases, sponsored by award PA# 19927 of the United States Department of Agriculture.

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Received: January 21, 2015 **Accepted: March 5, 2015**