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Twenty-Five Years of Clinical Flow Cytometry: AIDS Accelerated Global Instrument Distribution

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As a cell biologist, one experiences some ambivalence with an enigmatic sensation on the occasion of the silver jubilee of Clinical Cytometry. This significant milestone is celebrated with ominous feelings because of the remarkable symbiosis that was forged between the birth of a new clinical diagnostic tool and the emergence of one of the most devastating infectious diseases of the 20th century. It is less then 25 years ago that the first reports surfaced about clusters of aggressive opportunistic infections appearing mysteriously in young gay men in some metropolitan areas in the United States (1,2). The causative agent of the epidemic was unknown, yet the disease was well characterized within a very short time span as the acquired immunodeficiency syndrome (AIDS). The initial CDC definition of AIDS incorporated leukocyte subset numbers from peripheral blood. AIDS' defining condition included CD4 T-cell counts of 200 or less (3,4). Initially, in most immunology laboratories, immunophenotyping was performed with fluorescent microcopy. But quickly, the choice of assay method shifted to flow cytometry. As the world-wide apocalyptic devastation began, cellular immunologists engaged in research had at their disposal an arsenal of reagents and instruments just waiting for an opportunity for qualitative assessment of immunodeficiency. Therefore, in a very short time, instruments that could count T-helper cells were in great demand. The epidemic had profound impact throughout the Western world, but those initial human losses paled in comparison with the carnage AIDS delivered and still is delivering in resource-poor regions of the globe. It is estimated that in 2003, over 40 million individuals live with AIDS; about 10% of this population lives in South Africa.

Most of the time, significant scientific progress occurs in dull obscurity, but occasionally human curiosity prevails and the odd science story proceeds with significant fanfare. The discoverers of the structure of DNA did receive immediate accolades. However, in most instances of technical breakthroughs in diagnostics, the assessment of the day falls short of appropriate recognition. Invariably the press selects trivia to report as newsworthy science. For example, the postmortem analysis of the space shuttle disaster received phenomenal coverage. Polemical reports were bombarding us about how scientists were able to demonstrate that a piece of the foam debris at a certain velocity could have or could not have damaged critical ceramic tiles attached to the wing on that doomed and most unfortunate space vehicle. At the same time, this past July, a subcommittee of the International Union of Pure and Applied Chemists identified the 110th element. The new chart recognizes darmstadtium (Ds) as a unique element. It was formally added to the Periodic Chart. This latter event received virtually no publicity, while the significance of the finding is relatively easy to convey to the public, as we all learned about the elements while taking high school chemistry. The low profile coverage that is often associated with our discipline was altered forever 25 years ago. Events in cytology during the early 1980s were going to unfold in a most uneventful fashion. However, with a newly discovered incredibly devastating lethal disease on the horizon, the role of the underrated flow cytometer changed forever. Because reliable diagnosing of immunodeficiency was an essential requirement, clinical cytometry was born. With flow, rapid diagnosis of AIDS was possible as early as 1981 (1). Clinical immunophenotyping was possible just a few years after the discovery of the hybridoma technology. It opened the possibility for the production of mAbs with high affinity and avidity. Between 1978 and 1981, Milstein, Reinhertz, Schlossman, Goldstein, Janossy, and many others were diligently cranking out antibody-producing murine clones and were defining and assigning function-specific roles to markers on human T- and B-cells. Flow cytometry, a laser-based technology, was photogenic and there were no serological tests available to feature. Therefore, flow cytometry profited from seemingly spontaneous and persistent press coverage. The virtuosity and veracity of the investigators' productivity a quarter-century ago expedited research capacity in immunology beyond any expectations. Unfortunately, to date the regulatory role of various T-cell subsets has not been fully resolved. The scholarly struggle to gain more knowledge in this area, including oncology, has advanced, but it is far from over.

There is always a silver lining to any human catastrophe. The story of the AIDS pandemic is no exception. In this case, 25 years ago we had instrumentation to analyze peripheral blood cells, but there was no apparent widescale clinical application beyond differentiating various

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leukemia or lymphomas. We had an awesome new technology bundle that used laser power to recognize mAbs labeled with a variety of fluorochromes. The analysis included both intracellular and surface markers on leukocytes, but without relevant clinical utility. In medical diagnostics, progress is usually comparable to the work of the flow and ebb of the sea on shards of broken glass. Eventually, the sea will wash ashore a smooth mermaid's tear, a smooth-edged glass gem. Twenty-five years ago there was no time for mermaids' tears to be shed. Thanks to AIDS, practically overnight, the utility of flow cytometers became a clinical reality. A diagnostic technology was developed that harnesses simultaneously both intrinsic and extrinsic attributes of leukocytes (5). The rapid diagnosis of AIDS became possible years before the etiological agent for the disease was discovered (6).

The most extensive and incredible global public health project ever undertaken that involves the use of flow cytometry is just beginning to unfold. Unprecedented large-scale administration of antiretrovirals is on its way. All this dramatic activity is occurring in resource-poor regions, on three different continents. For example, in Brazil alone there are 85 flow cytometers in the field, supported by external quality assessment programs to monitor antiretroviral drug administration. Effective triple therapy is available at $150 per year, when it is over $10,000 per year in most of Europe and North America. It is anticipated that the cost of CD4 T-cell enumeration will drop from the average $50 to under $5 in many resource-poor regions within the next 12 months. A greater than one log cost reduction of a diagnostic test will have profound effect on infectious disease control in a global context. The ultimate aim is to take advantage of this momentum and develop more advanced flow cytometers as the heart of a new universal multiplexed and multitasking diagnostic platform (7,8). The next generation of flow-based modular platforms will open an era of affordable and comprehensive diagnostics by telemetry in the global village. Cytomics will take us to uncharted dimensions of integrated diagnostic medicine of the 21st century in a Jules Vernean vision. Stay tuned for the next 25 years.

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