Review Article

The origin of some laboratory medicine milestones

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Abstract: Although the real contribution of laboratory diagnostics to the managed care remains largely debated, it is now unquestionable that both the development and introduction into clinical practice of many laboratory tests have virtually revolutionized clinical practice. This article is hence aimed at presenting and discussing the original discovery of some laboratory analyses which have profoundly revolutionized science and medicine, and that are now largely used in daily practice for managing many million patients worldwide without that some of us would probably know how, when and why this has initially occurred. These tests, which are just arbitrarily selected but probably paradigmatic examples, include cardiac troponins, procalcitonin, glycated hemoglobin, prothrombin time and carcinoembryonic antigen (CEA). What can be learnt from the historical overview of these cases is that although a strict relationship can be not always found between the outcome of the discovery process and the original intention that drove it forward, scientists must always critically analyze their findings, reflecting on the potential significance, establishing a link with previous knowledge and finally driving their (occasionally serendipitous) discovery into maximized benefits for science and medicine. It is virtually undeniable that studying the history of laboratory medicine is essential, since it will help understanding our past, and will serve as a guide for the present and for the future.

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Introduction

Laboratory medicine is conventionally depicted as a medical discipline committed to generating clinical information by analyzing the concentration, composition and/or structure of analytes in different biological fluids (1). Along its relatively long history, laboratory medicine has provided, and is still providing, an irreplaceable contribution to the clinical decision making across many diagnostic domains, thus encompassing screening, diagnosis, prognostication and therapeutic monitoring of the vast majority of human pathologies.

Although the real contribution of diagnostic testing to the managed care remains largely debated (2), it is now unquestionable that both the development and introduction into clinical practice of many laboratory tests have virtually revolutionized clinical practice. As for the discovery of penicillin by Fleming in 1928 (a petri dish with a *Staphylococcus* culture was accidentally destroyed by contaminating *Penicillium* mold spores) (3), the invention, validation and clinical acknowledgement that some laboratory tests produce a strong impact on clinical reasoning and managed care has been sometimes a serendipitous and involuntary event. In other circumstances the development of some laboratory analyses has instead been the consequence of a complex reasoning, supported by strong underlying biochemical and biological evidence, which has then allowed transferring research findings from the bench to the bedside. Irrespective of how the clinical usefulness of some tests has been firstly identified, the following parts of this article are aimed to present and discuss the original discovery of some laboratory analyses which have profoundly revolutionized science and medicine, and that are now largely used in daily practice for managing many million patients worldwide, without that some of us would probably know how, when and why this has initially

Table 1 Laboratory medicine milestones

Test	Pathology; indication	Number of patients (worldwide)	Discovery (year)	Clinical usefulness (year)
Cardiac troponins	Acute myocardial infarction; diagnosis	33 million/year	1965	1983
Glycated haemoglobin	Diabetes; diagnosis and therapeutic monitoring	422 million	1975	1981
Procalcitonin	Sepsis; diagnosis and therapeutic monitoring	30 million/year	1968	1971
Prothrombin time	Anticoagulation; therapeutic monitoring	75 million	1935	1941
Carcinoembryonic antigen	Colorectal cancer; diagnostics (monitoring)	2 million	1965	1965

occurred. These tests, which are just arbitrarily selected but probably paradigmatic examples, include cardiac troponins, procalcitonin, glycated hemoglobin, prothrombin time and carcinoembryonic antigen (CEA) (*Table 1*).

Cardiac troponins for diagnosing acute myocardial infarction

According to the recent, universally agreed guidelines for diagnosing acute myocardial infarction, the measurement of cardiac troponins is now a virtually irreplaceable step for early and accurate identification of cardiac injury (4).

The structure and function of the troponin complex, which has been originally called "'tropomyosin-like protein", was first characterized by Ebashi and Kodama, in 1965 (5), as a new muscular protein complex promoting the aggregation of tropomyosin. The very first study revealing the potential clinical usefulness of measuring cardiac troponins in the diagnostics of acute myocardial infraction was then published (in form of an abstract) by Cummins and Auckland, in 1983 (6). Briefly, the authors measured cardiac troponin I (cTnI) by means of a specific radioimmunoassay with no cross-reactivity with the muscular counterpart (mTnI), and found that cTnI values were comprised between 20–550 ng/mL in patients with acute myocardial infarction, whilst those of a normal reference population were always <13 ng/mL. The kinetics after acute myocardial infarction was also found to be consistently different from that of creatine kinase isoenzyme MB (CK-MB), since cTnI values remained elevated for up to 6 days post-infarction compared to a much shorter time for CK-MB. This preliminary report was then followed, 4 years later, by a full-length article published in the *American Heart Journal* (7). In this more thorough report, the authors not only described the biochemical characteristics of cTnI favouring the development of specific antibodies, but also extended previous clinical findings in a large patient population. Notably, cTnI values were measured with the same in-house developed radioimmunoassay and were found to be significantly and constantly higher in patients with acute myocardial infarction (mean value, 112 ng/mL; range, 20–550 ng/mL) than in control subjects (mean value, <10 ng/mL; range, all <10 ng/mL), and than in those with skeletal muscle injury (mean value, <10 ng/mL; range, all <10 ng/mL) and non-cardiac chest pain (mean value, <10 ng/mL; range, <10–17 ng/mL). The measurement of cTnI outperformed the diagnostic performance of CK-MB throughout all these categories of patients. Additional data were also presented on the comparative CK-MB and cTnI kinetics after ischemic cardiac injury, with the former enzyme returning to baseline values after ~3 days compared to 6–8 days for cTnI.

Procalcitonin for diagnosing sepsis

The discovery that the hormone calcitonin was synthesized from a higher molecular weight precursor (which was then called "procalcitonin") has been originally published by Fernando Moya and co-authors, in 1975 (8). Albeit the use of procalcitonin for diagnosing and even for monitoring sepsis is now almost unquestionable (9), the first evidence on the clinical usefulness of this biomarker was mostly indirect and partially involuntary. In 1981 Wagner and colleagues published a short but interesting report in *Lancet* (10), which described the cases of nine women

with the so-called "toxic shock syndrome", most likely caused by *Staphylococcus aureus*. Notably, all these patients displayed sustained hypocalcaemia, hypophosphatemia and low albumin values, which were accompanied by remarkably increased calcitonin values (i.e., between 177–10,735 pg/mL; reference range, <135 pg/mL). Although these important metabolic variations, which included serum calcitonin increase, were attributed to the effect of the pathogen itself (i.e., *Staphylococcus aureus*) or its toxins, the precise underlying biological mechanisms remained obscure for long.

The very first evidence that procalcitonin measurement is a useful tool in diagnosing bacterial sepsis was hence published 12 years later, by Assicot and colleagues, in *Lancet* (11). Briefly, the authors developed a monoclonal immunoradiometric assay for calcitonin precursors, including procalcitonin, and used this method for measuring serum procalcitonin in 79 infants and children with suspected infections. Overall, consistently high serum procalcitonin values were found in subjects with severe bacterial infections (range, 6–53 ng/mL) compared to those with no infection (all <0.1 ng/mL). Interestingly, modestly increased procalcitonin values were also observed in patients with peripheral or local bacterial infections (range, 0.1–1.5 ng/mL). Finally, the kinetics of procalcitonin was found to be strongly correlated with infective complications and septic episodes in intensive care patients, thus paving the way for the routine use of this biomarker for diagnosing and therapeutic monitoring of severe infections.

Glycated haemoglobin for diagnosing and monitoring diabetes

The routine measurement of glycated hemoglobin (HbA1c) for monitoring diabetes control over the previous 2 to 3 months has been for long used around the globe. Most recently, however, an elevated value of HbA1c has also been introduced as one of the leading criteria for diagnosing diabetes mellitus (12).

It may seem strange or even paradoxical but, as for procalcitonin, the original discovery of the clinical usefulness of HbA1c can be considered mostly casual. In 1968, Rahbar published a short note *in Clinica Chimica Acta* (13), describing the evidence of what he originally called "abnormal fast moving hemoglobin fraction" in the blood of two diabetic patients, which was then identified as being the formerly known hemoglobin variant "HbA1c". An additional study was planned by Rahbar, and this additional fraction could then be detected in the blood of other 47 diabetic patients. Rahbar concluded that the nature of this abnormality was unclear, so that further work was needed. Further insights were hence published 3 years later by Trivelli and colleagues in *New England Journal of Medicine* (14). Briefly, the authors studied 100 diabetic subjects and 20 controls, and found that the HbA1c values were nearly double in diabetics (range, 6–10%) than in controls (range, 3.3–3.5%). Notably, the authors only concluded that the enhanced proportion of HbA1c in diabetes was another example of increased glycoproteins in this condition, without actually guessing that their finding had paved the way to the routine use of this biomarker in millions of diabetics.

The prothrombin time for monitoring warfarin therapy

Anticoagulant therapy is now widely used for preventing and/or treating a kaleidoscope of human prothrombotic and thrombotic conditions, such as venous thromboembolism, cardiovascular disease and atrial fibrillation, among others. Although the diffusion of new direct anticoagulant agents [i.e., direct oral anticoagulants (DOACs)] is constantly expanding, warfarin remains the leading anticoagulant agent worldwide (15).

The narrow therapeutic range is the most challenging aspect in warfarin administration, wherein its anticoagulant potency must be regularly monitored to prevent under- or over-coagulation, which may then expose the patients to a substantially increased risk of thrombosis and bleeding, respectively. Warfarin monitoring is now universally accomplished by using a simple and inexpensive laboratory test, called prothrombin time (PT). The first version of this test has been originally published by Quick in 1935 (16), and has then been refined by Owren and Aas years later (17). Reliable evidence that this test could be used for monitoring warfarin therapy was first published by Campbell and colleagues, in 1941, in *Journal of Biological Chemistry* (18). Interestingly, the authors fed rabbits with a spoiled sweet clover hay which had been responsible for the hemorrhagic sweet clover disease (only lately warfarin was identified as the responsible agent), and showed that the ratio of clotting time before and after feeding represented a reliable index of the amount of prothrombin inactivated by the anticoagulant agent. This hence represented the main underlying assumption for then using the PT for monitoring millions of patients undergoing warfarin therapy.

CEA

The original discovery of the CEA can be dated back to the 1965, when Gold and Freedman published the results of their research in *Journal of Experimental Medicine* (19). Their studies were basically aimed at using absorption and immune tolerance techniques for demonstrating the presence of cancer-specific antigens in serum of animals immunized with human colorectal cancer preparations. In the very first study, Gold and Freedman demonstrated that colorectal adenocarcinoma contained at least two specific cancer antigens, which were absent in normal tissue, and were capable to stimulate the production of cancer-specific antibodies in animals. Even more interestingly, the same authors planned to assess as to whether these two antigens could also be identified in other adult human tissues as well as in human embryonic and foetal tissues in an ensuing study (20). The important finding of this second research was that these antigenic constituents appeared specifically expressed by malignant cancer cells of the human digestive system, were absent from non-cancerous adult tissues, but could still be identified in foetal liver, gut, and pancreas (at 2–6 weeks of gestation age). These molecules were hence finally called "carcinoembryonic antigens" of the human digestive system. Quite interestingly, the authors' conclusions laid the groundwork for all future colorectal cancer research, whereby it was suggested that these CEAs may be seen as cellular constituents which are repressed during digestive system differentiation, but may then reappear during malignant cell transformation for derepressive-dedifferentiation.

Conclusions

In his celebrated "*De Oratore*", a dialogue written by Cicero in the year 55 BC, the famous Latin poet concluded that "*historia magistra vitae est*" (i.e., "history is life's teacher"). This actually means that studying history remains essential, since it will help understanding our past, and will serve as a guide for designing our present and our future. There are many lessons than can be learnt from the past, and which can help us developing a brighter future, since history is made of cycles and counter cycles. Laboratory medicine makes no exception to this rule. The five examples discussed in this article, representing laboratory tests that are now used for managing millions of patients worldwide, lead the way to formulate some important reflections. Although the discovery and development of a new test can be the obvious consequence of a logical reasoning, based on a strong biochemical and biological background (such as in the case of cardiac troponins or CEA), in other cases some laboratory tests have been developed after a serendipitous event, mostly occurred by chance (such as in the cases of procalcitonin and HbA1c). This clearly demonstrates that a strict relationship can be not always found between the outcome of a discovery process and the original intention that drove it forward. Irrespective of the fact that the development of a new test has been the outcome of a "causal" or "casual" process, the scientist must hence be always capable to analyze their findings, reflecting on the potential significance, establishing a link with previous knowledge and finally driving their (occasionally serendipitous) discovery into maximized benefits for science and medicine.

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Footnote

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References

1. Lippi G. The irreplaceable value of laboratory diagnostics: four recent tests that have revolutionized clinical practice.

EJIFCC 2019;30:7-13.
2. Hallworth MJ. That '70%' claim again. Ann Clin Biochem 2018;55:517-8.
3. Fleming A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. 1929. Bull World Health Organ 2001;79:780-90.
4. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth Universal Definition of Myocardial Infarction (2018). Circulation 2018;138:e618-51.
5. Ebashi S, Kodama A. A new protein factor promoting aggregation of tropomyosin. J Biochem 1965;58:107-8.
6. Cummins P, Auckland ML. Detection of myocardial cell injury: evaluation of a novel cardiac specific radioimmunoassay. Eur Heart J 1983;4:78.
7. Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J 1987;113:1333-44.
8. Moya F, Nieto A, R-Candela JL. Calcitonin biosynthesis: evidence for a precursor. Eur J Biochem 1975;55:407-13.
9. Lippi G, Cervellin G. Procalcitonin for diagnosing and monitoring bacterial infections: for or against? Clin Chem Lab Med 2018;56:1193-5.
10. Wagner MA, Batts DH, Colville JM, et al. Hypocalcaemia and toxic shock syndrome. Lancet 1981;1:1208.
11. Assicot M, Gendrel D, Carsin H, et al. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515-8.
12. American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care 2018;41:S13-27.
13. Rahbar S. An abnormal hemoglobin in red cells of diabetics. Clin Chim Acta 1968;22:296-8.
14. Trivelli LA, Ranney HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971;284:353-7.
15. Lippi G, Mattiuzzi C, Cervellin G, et al. Direct oral anticoagulants: analysis of worldwide use and popularity using Google Trends. Ann Transl Med 2017;5:322.
16. Quick AJ, Stanley-Brown M, Bancroft FW. A study of the coagulation defect in hemophilia and in jaundice. Am J Med Sci 1935;190:501-10.
17. Owren PA, Aas K. The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scand J Clin Lab Invest 1951;3:201-8.
18. Campbell HA, Smith WK, Roberts WL, et al. Studies on the hemorrhagic sweet clover disease: II. The bioassay of hemorrhagic concentrates by following the prothrombin level in the plasma of rabbit blood. J Biol Chem 1941;138:513-27.
19. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 1965;121:439-62.
20. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965;122:467-81.

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