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Myocarditis diagnosis: From light microscope to molecular analysis and cardiac magnetic resonance

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Telling story of myocarditis is characterized by discoveries and inventions. The invention of the microscope opened new avenues in medicine, with the observation of myocardial inflammation by Carl Ludwig Alfred Fiedler. Rudolph Virchow discovered that cells are the elementary units. Karl Albert Ludwig Aschoff first reported rheumatic pancarditis. Gilbert Dallford found enterovirus in the faeces of children, who died suddenly in the village of Coxsackie. Werner Forssmann entered in his own right ventricle with a urologic catheter via the left radial vein. Endomyocardial biopsy, via the femoral or jugular veins, made possible to take away myocardial samples *in vivo*, for diagnosis of myocarditis or cardiac rejection of transplanted heart. The invention of polymerase chain reaction by Kary Mullis allowed to achieve diagnosis of concealed infections and genetically determined cardiomyopathies. Myocarditis, a significant cause of sudden death, was found to be frequently ascribed to viruses. Cytotoxicity of Coxsackievirus B was proved to consist on released protease 2, encoded by virus genome and cleaving dystrophin. RNA (Coxsackie) and DNA (adenovirus) viruses share a common receptor. Cardiac magnetic resonance revealed to be sensitive and specific in the diagnosis of myocarditis by detecting myocardial oedema. However, it is unable to establish the histotype. The onset of myocarditis may be fulminant; however, extracorporeal membrane oxygenation, invented by Robert Bartlett, allows heart rest, while replacing cardiac contractility. High rates of survival have been achieved, probably because of mild myocardial damage.

The eyes are not enough to explore the nature. The invention of light microscope by Robert Hooke (1635–1703) in 1665[1] opened new avenues in medicine with 'micrografia', allowing to observe 'minute bodies' (*Figure 1*).

With the employment of microscope, Rudolph Virchow (1821–1902) discovered that cells are the elementary units of organs. Moreover, looking under the microscope, it was possible to discover infective microorganisms, like Robert Koch (1843–1910) did with mycobacterium of tuberculosis.

In 1800, Carl Ludwig Alfred Fiedler (1835–1921) (*Figure 2A*)[2] first reported autopsy cases of people who died by heart failure in the absence of coronary, valve, and pericardial diseases. By histology and microscope, he detected inflammatory cells in the myocardium, which he named acute interstitial myocarditis, an isolated phenomenon sparing the other organs and consisting of lymphocytes and giant cells as inflammatory infiltrates (*Figure 2B, C*). The eponym giant cell myocarditis was then employed in the literature.

In 1900, Karl Albert Ludwig Aschoff (1866–1942) (*Figure 3A*)[3] observed at the microscope granulomatous inflammatory 'bodies' (called rheumatic nodules), with owl eye cells (*Figure 3B*), in the setting of pancarditis (valves, pericardium, and myocardium inflammatory involvement).

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Figure 1 Robert Hooke (1635–1703) invented the light microscope in 1665, to observe 'minute bodies'.

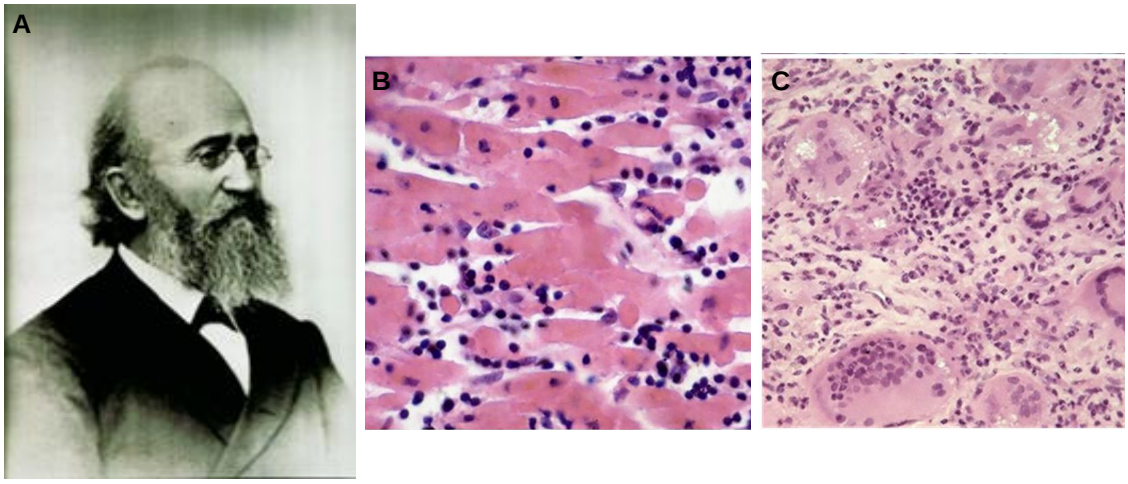

Figure 2 (*A*) Photo of Carl Ludwig Alfred Fiedler (1835–1921). Histotypes of interstitial myocarditis at microscope: lymphocytic (*B*) and giant cell (*C*) infiltrates.

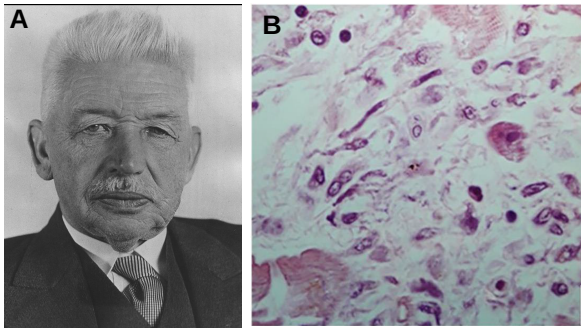

Figure 3 (*A*) Photo of Karl Albert Ludwig Aschoff (1866–1942). (*B*) Rheumatic nodule with owl eye cells at microscope.

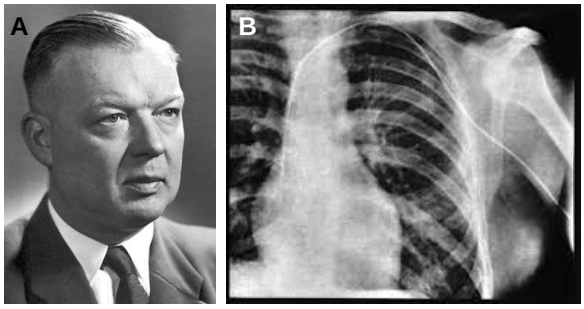

Figure 4 (*A*) Photo of Werner Forssmann (1904–1979). (*B*) A urological catheter, introduced in the left radial vein, reaches his right ventricle.

Carlos Chagas (1879–1934) in 1909 in Brazil described myocarditis by the protozoan *Trypanosoma cruzi*.

In 1929, Mitchell Bernstein first reported sarcoidosis myocarditis, in the setting of a systemic non-infective inflammatory disease, most probably immune, characterized by non-caseous giant cells granuloma. Heart involvement by sarcoidosis has been confirmed later.

Gilbert Dallford (1900–1979) in 1948 discovered enterovirus in the faeces of children who died with

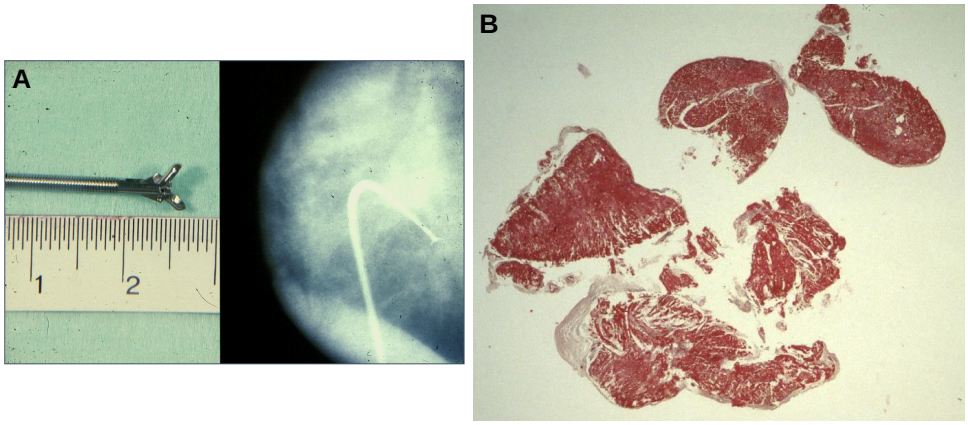

Figure 5 (*A*) Endomyocardial biopsy catheter with bioptome via femoral vein was invented in 1974 by Richardson at the King's College in London. (*B*) Endomyocardial biopsy with multiple samples.

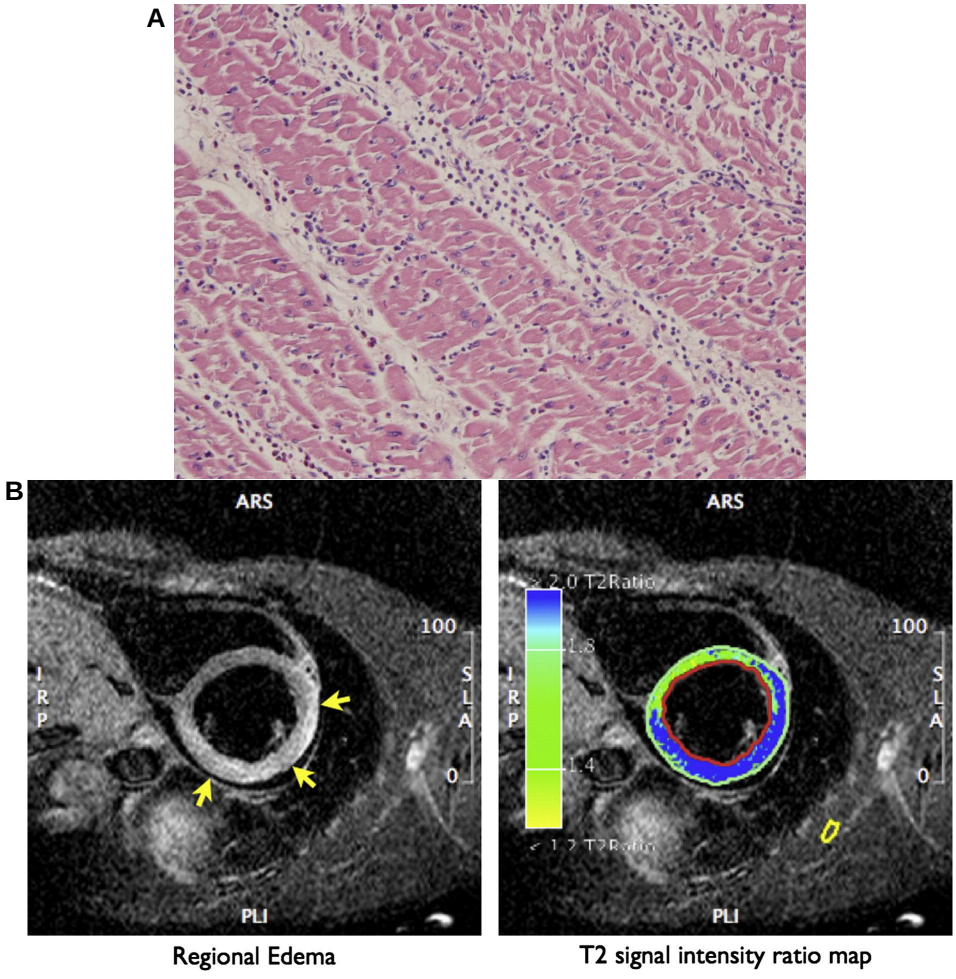

Figure 6 (*A*) Interstitial myocarditis with oedema, in the absence of myocardial necrosis. (*B*) T2-weighted short-axis view of myocardial oedema by cardiac magnetic resonance in acute myocarditis.

congestive heart failure by myocarditis diagnosed at autopsy, in the village of Coxsackie which gave the name to the disease.[4]

In vivo diagnosis of myocarditis became possible, thanks to the invention of cardiac catheterism by the German urologist Werner Forssmann (1904–1979) (*Figure 4A*), who entered his own right ventricle with a urological catheter through left radial vein (*Figure 4B*).[5] He was awarded with Nobel Prize in 1956, together with André Frédéric Cournand and Dickinson W. Richards from New York.

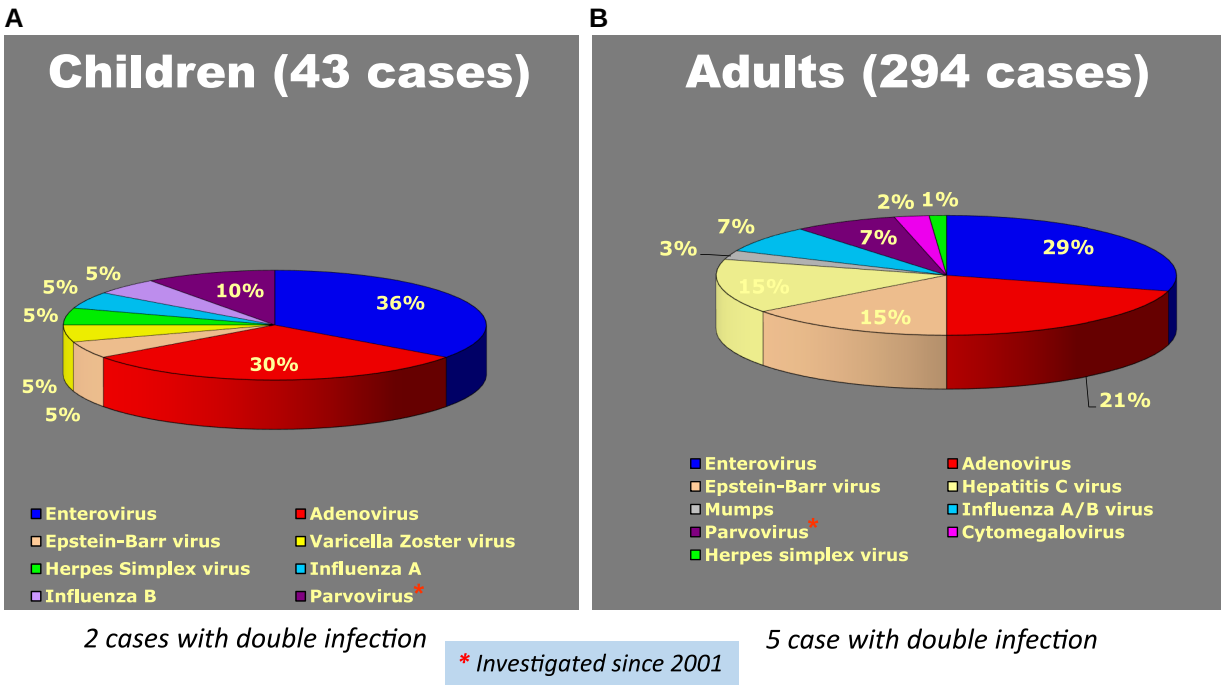

Figure 7 Molecular investigation of myocarditis, time interval 1996–2005, in the Veneto Region, Italy. Enteroviruses were the main cause of viral myocarditis, both in children (*A*) and adults (*B*).

Transvenous endomyocardial biopsy was invented by the Japanese Sakakibara and Konno in 1962. It was then possible to get myocardial samples by endomyocardial biopsy from the right ventricle, with a catheter introduced through the femoral vein via the inferior vena cava by Richardson in 1974 at the King's College Hospital in London with a bioptome, able to take away multiple samples (*Figure 5*).

With the endomyocardial biopsy, several different histotypes of myocarditis were detected.

Another approach through the right jugular vein was used by Margaret Billingham in Stanford for monitoring cardiac rejection in heart transplantation. Cardiac rejection consisted of aggressive myocardial inflammatory cell infiltrates, similar to lymphocytic myocarditis.

In the 1985, Dallas criteria were put forward by the Society for Cardiovascular Pathology for the diagnosis of myocarditis including both inflammatory infiltrates and myocardial necrosis.[6] However, fulminant myocarditis may disclose only interstitial inflammatory infiltrates with oedema, in the absence of myocardial necrosis (*Figure 6A*). Subsequently, Baughman dead the Dallas criteria.[7] Oedema is nowadays detected by cardiac magnetic resonance, quite sensitive and specific for the diagnosis of myocarditis (*Figure 6B*).[8]

The invention in 1993of polymerase chain reaction (PCR), by Nobel Prized Kary Mullis (1944–2019), allowed to achieve the diagnosis of concealed diseases, both infective and genetically determined.[9] Polymerase chain reaction is now considered the gold standard for the diagnosis of viral myocarditis both *in vivo* and at autopsy (*Figure 7*).

In 1986, Bowles *et al*. first applied the technique of *in situ* hybridization, able to detect intracellular Coxsackie virus in myocardial biopsy samples of patients with acute myocarditis or dilated cardiomyopathy.

Rudolph Virchow, while discussing with Karl von Rokitansky in 1894 at a meeting in Rome, predicted: '… Any anatomic modification is material, but is any material modification anatomic? Why not molecular? Can a profound molecular modification occur in the setting of an apparently normal structure? These modifications belong more to physiology than to anatomy, they are functional-dynamic… the method of investigation will never be morphological'.

In the time interval 1996–2005, within the Cardiac Registry of Cardiovascular Diseases of the Veneto Region Italy, a molecular investigation by PCR was systematically carried out in endomyocardial samples of both children and adults myocarditis. Enterovirus, Coxsackie included, resulted to be the most frequent virus, both in children (*Figure 7A*) and adults (*Figure 7B*).

Follow-up showed that enteroviruses were associated with a better survival. Myocarditis resulted to be a frequent cause of sudden death in the young, with enterovirus being the most frequent cause of viral myocarditis at molecular autopsy.[10]

Fibrosis is the outcome of myocardial necrosis in myocarditis, with severe congestive heart failure requiring heart transplantation.

The cardiac damage may be direct by virus or autoimmune mediated (humoral or cell rejection).

As far as direct cardiomyocyte cytotoxicity of Coxsackie B, Badorf *et al*. in 1999 discovered the mechanism consisting on release of protease 2A, encoded by virus genome which cleaves dystrophin.[11,12]

Moreover, He *et al*. in 2021 demonstrated that Coxsackievirus (RNA) and adenovirus (DNA) share a common receptor (CAR).[13]

The clinical presentation of myocarditis may be fulminant, but not necessarily fatal.[14] Spontaneous

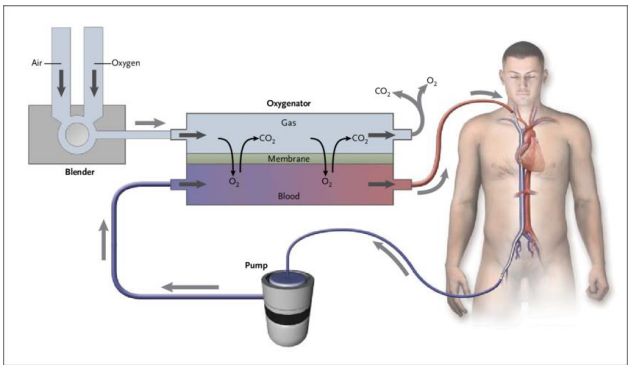

Figure 8 In 1970, Robert Bartlett (1939) invented extra corporeal membrane oxygenation, which allows the native heart to rest and to recover contractile-respiratory functions.

Table 1 High rate of survival from fulminant myocarditis treated with extra corporeal membrane oxygenation, both in children and adults

Contractile recovery and rate of survival by extra corporeal membrane oxygenation in fulminant myocarditis	
Duncan *et al*. *J Thorac Cardiovasc Surg*, 2001	80% (children)
Teel *et al*. *J Pediatr*, 2011	80% (children)
Didle *et al*. *Crit Care Med*, 2015	61% (adults)
Lorusso *et al*. *Ann Thorac Surg*, 2016	71% (adults)

resolution may occur with the support of extracorporeal membrane oxygenation, invented in 1970 by Robert Bartlett (1939) (*Figure 8*). It allows temporary native heart rest, by supplying cardiac contractile and respiratory functions. A contractility recovery, most probably due to mild myocardial damage, has been proven by significant long distance survival, both in children and adults with fulminant myocarditis (*Table 1*).

Coxsackievirus vaccination has been proven to be effective in experimental animals, but not yet employed in the clinical setting.[15,16]

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Conflict of interest: none declared.

Data availability

No new data were generated or analysed in support of this research.

References

1. Hooke R. *Micrographia, or, Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses with Observations and Inquiries Thereupon*. In: Martyn J, Allestry J. London, UK: Royal Society; 1665.
2. Fiedler A. Uber akute interstitielle Myokarditis. *Zentralblatt Inn Med* 1900;**21**:212.
3. Aschoff KAL. Zur Myocardiitisfrage. *Verhandlungen Dtsch Pathol Ges Stuttg* 1904;**8**:46–53.
4. Dalldorf G, Sickles GM. An unidentified, filtrable agent isolated from the feces of children with paralysis. *Science* 1948;**108**:61–62.
5. Forssmann W. Die Sondierung des rechten Herzens [probing of the right heart]. *Klin Wochenschr* 1929;**8**:2085–2087.
6. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ Jr *et al*. Myocarditis. A histopathologic definition and classification. *Am J Cardiovasc Pathol* 1987;**1**:3–14.
7. Baughman KL. Diagnosis of myocarditis: death of Dallas criteria. *Circulation* 2006;**113**:593–595.
8. Friedrich MG, Sechtem U, Schulz-Menger J, Holmvang G, Alakija P, Cooper LT *et al*. Cardiovascular magnetic resonance in myocarditis: a JACC white paper. *J Am Coll Cardiol* 2009;**53**:1475–1487.
9. Mullis K.B, Erlich H.A, Gelfand D.H, Horn G, Saiki R.K. Process for Amplifying, Detecting, and/or-Cloning Nucleic Acid Sequences. U.S. Patent 4,683,195, 1986.
10. Basso C, Calabrese F, Angelini A, Carturan E, Thiene G. Classification and histological, immunohistochemical, and molecular diagnosis of inflammatory myocardial disease. *Heart Fail Rev* 2013;**18**:673–681.
11. Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE *et al*. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med* 1999;**5**:320–326.
12. Andréoletti L, Ventéo L, Douche-Aourik F, Canas F, Lorin de la Grandmaison G, Jacques J *et al*. Active Coxsackieviral B infection is associated with disruption of dystrophin in endomyocardial tissue of patients who died suddenly of acute myocardial infarction. *J Am Coll Cardiol* 2007;**50**:2207–2214.
13. He Y, Chipman PR, Howitt J, Bator CM, Whitt MA, Baker TS *et al*. Interaction of Coxsackievirus B3 with the full length Coxsackievirus-adenovirus receptor. *Nat Struct Biol* 2001;**8**:874–878.

14. Lieberman EB, Hutchins GM, Herskowitz A, Rose NR, Baughman KL. Clinicopathologic description of myocarditis. *J Am Coll Cardiol* 1991; **18**:1617–1626.
15. Zhang L, Parham NJ, Zhang F, Aasa-Chapman M, Gould EA, Zhang H. Vaccination with Coxsackievirus B3 virus-like particles elicits humoral immune response and protects mice against myocarditis. *Vaccine* 2012;**30**:2301–2308.
16. Yun SH, Lee WG, Kim YC, Ju ES, Lim BK, Choi JO *et al*. Antiviral activity of Coxsackievirus B3 3C protease inhibitor in experimental murine myocarditis. *J Infect Dis* 2012;**205**:491–497.