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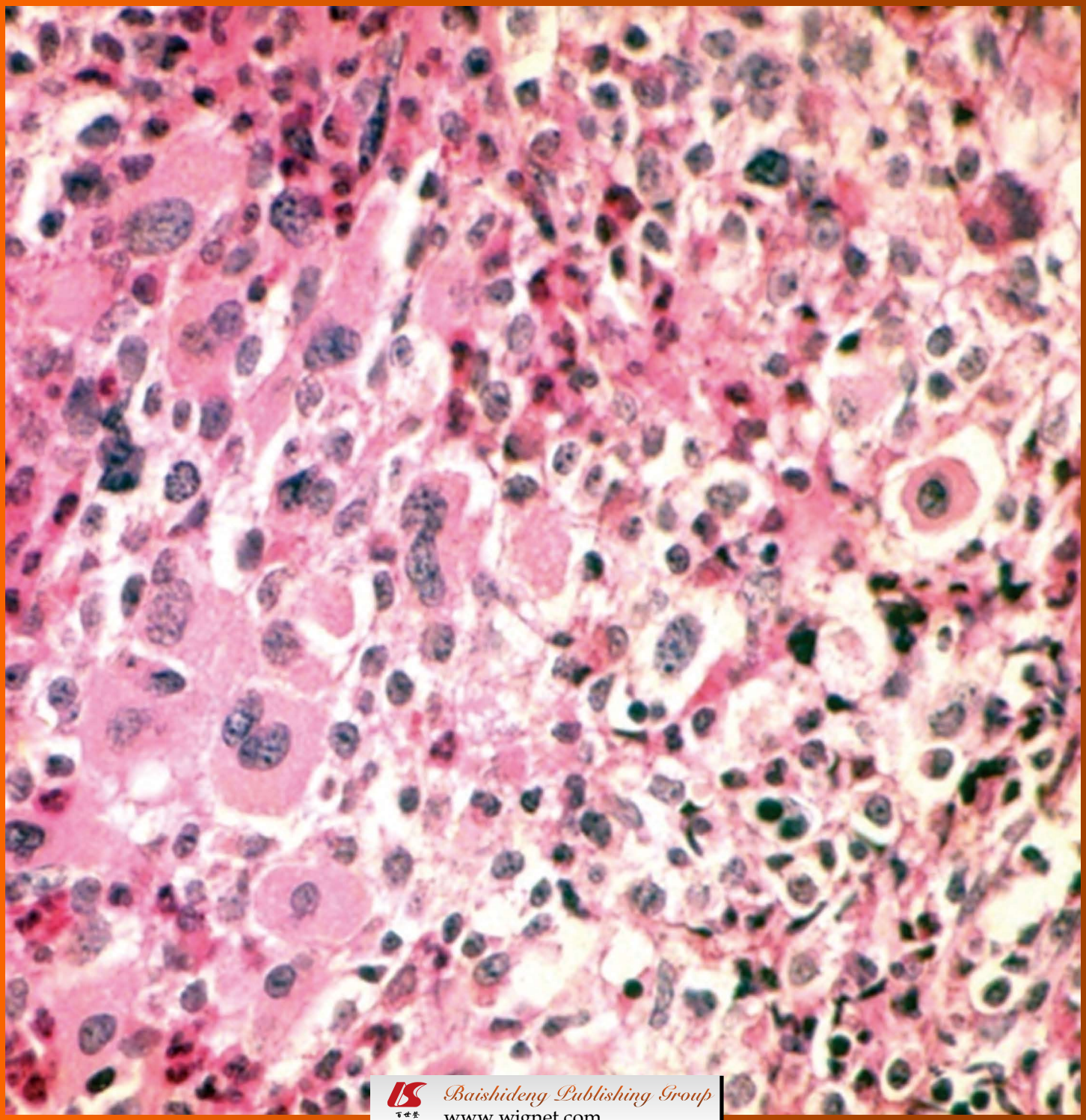

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World Journal of Hematology

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AIM AND SCOPE

World Journal of Hematology (*World J Hematol*, *WJH*, online ISSN 2218-6204, DOI: 10.5315) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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REVIEW

First contributors in the history of leukemia

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Abstract

While the modern era of leukemia chemotherapy began recently, the recognition of leukemias has been mainly recorded in the second part of the nineteenth century. This brief historic review reports the first descriptions of the disease and the major advances in its history from its roots to the beginning of the twentieth century. Although most treatments for leukemia were ineffective until the middle of the twentieth century, it seemed of interest to review some pertinent exemples of the evolution in the knowledge of this disease (relied upon chronology as an organizing framework, while stressing the importance of themes), since our current knowledge about leukemia is still mainly based on the first accounts of scientific and medical discovery. Early in the nineteenth century, a small number of cases of patients with uncommon or peculiar alterations of the blood were published. Of the cases, four might suggest symptoms of chronic leukemia. The first published case was the detailed report prepared by John Hughes Bennett in the "*Edinburgh Medical and Surgical Journal*" October 1845. Leukemia gradually became accepted as a distinct disease and published case reports grew in number. Concomitantly, clinical and pathological description of the disease became more detailed.

Key words: History; Leukemia; Discovery; Hematology

Core tip: Towards the end of the nineteenth century, a clearer definition of the classification of leukemia had been established leading to different subtypes. These well-defined subtypes of leukemia were used for the development of effective chemotherapy, which has represented the most important advance in leukemia research during the past half century.

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INTRODUCTION

The oldest description of cancer was discovered in Egypt and dates back to approximately 1600 BC. The origin of the word "cancer" is credited to the Greek physician Hippocrates (460-370 BC). Hippocrates noticed that blood vessels around a malignant tumor looked like the claws of crab[1]. He named the disease karkinos (the Greek name for crab) to describe tumors that may or may not progress to ulceration. The credit for the discovery of leukemia goes to the ancient Greeks, who recognized this blood disease way back in the 4th or 5th century BC. However, the literature until AD 500 revealed no evidence of blood malignancies. Accounts and traces of leukemia in the literature are of relatively recent origin. It is obvious that modern knowledge about leukemia owes a debt to numerous European physicians.

In this brief historical review, we examined the major initial advances in the history of leukemia and the main characters involved in the first descriptions. Although "discovery" could not be attributed to one unique observation and that accurate knowledge and effective treatment of leukemia were not available until the middle of the twentieth century, it seemed of interest to review some pertinent examples of the first descriptions and

advances in this disease. Targeted more specifically at the young hematologist and the general educated reader, this review aims to communicate accounts of scientific and medical discovery and initial practice in this disease. It relies upon chronology as an organizing framework, while stressing the importance of themes.

FIRST STUDIES OF BLOOD

Naked-eye inspection of blood by phlebotomy was practiced far back to ancient times. The ancients readily recognized the importance of blood as a life-giving substance, believing it to hold the body's vital force. Hebrews back to the patriarchal age maintained that blood was the seat of the soul and demanded through the Mosaic laws that it be drained before an animal was prepared as food. The Romans drank the blood of their enemies, thinking it would confer on them the courage of their vanquished foes. The scientific study of blood had to wait for the invention of the microscope. While magnifying lenses were known to the monastic scholar and natural historian Roger Bacon (1214-1294), lenses of sufficient quality for scientific use were not available for another three centuries[2]. Invention of the compound microscope around 1590 by Hans and Zacharias Janssen made possible the examination of the content of the blood[3]. In 1658, the Dutch naturalist, Jan Swammerdam (1637-1680) was the first person to observe red blood cells under the microscope[4]. Another Dutch microscopist Anton van Leeuwenhoeck (1632-1723) who developed a number of primitive compound microscopes, described the size and shape of "red corpuscules" and rendered the first illustration of human red blood cells in 1695[5]. The white blood cells ("*the globuli albicantes*") were first noted by Joseph Lieutaud (1703-1780)[6], some 20 years before William Hewson (1739-1774) first described the lymphocyte[7]. The earliest reports of leukemia were therefore made by pathologists who recognized early in the nineteenth century, first by gross observation and later through microscopy, that the blood was composed predominantly of white cells, but the evidence provided in the publications was insufficient to support a definite diagnosis of leukemia[8-12]. This is only in the 19th century that several European physicians noticed that quite a few of their patients suffered from abnormally high levels of white blood cells.

FIRST DESCRIPTIONS OF LEUKEMIA

Similar to most discoveries in medicine, it is not clear who first actually discovered leukemia. Early in the nineteenth century, a small number of cases with uncommon alterations of the blood were published[13]. The earliest report of the illness is generally considered to have been made by Velpeau[12] (Figure 1). Velpeau[12] reported a case of a 63-year-old woman who had fever, a swollen stomach, as well as being generally weak. At autopsy, she was found to have an enormous spleen (twenty times larger than normal) and whose blood was "thick like gruel such that one might have asked if it were not rather laudable pus, than blood". However, at the time no official name was assigned to the condition and the scant evidence provided in the publication was insufficient to support a definite diagnosis of leukemia. The disease might well have been first observed by Alfred Donné of Paris, the founder of French clinical microscopy (Figure 2). In 1839, Dr. Barth and Dr. Chomel requested his consultative services for a woman patient aged 44 suffering from a painless left-sided abdominal tumor filling the left side of the abdomen. After requesting a specimen of blood for examination, Donné indicated that the large excess of white blood cells which might resemble purulent matter, was not in fact such and concluded that the patient presented with a new disease[14]. Donné wrote Barth as follows: "The blood you sent me shows a remarkable and most conspicuous change. More than half of the cells were mucous globules. You know that normal blood contains three types of cells: (1) red cells, the essential cells of the blood; (2) white cells or mucous cells; and (3) the small globules. It is the second variety which dominates so much, that, one wonders, knowing nothing about the clinical course, whether this blood does not contain pus. As you know the pus cells cannot yet be differentiated with accuracy from mucous cells"[15]. In a later case, he reported far more accurately on leukemia and wrote the following text for a chapter entitled "De l'altération des globules blancs" which appeared in his book "Cours de microscopie complémentaire des études médicales": "There are conditions in which white cells seem to be in excess in the blood. I found this fact so many times, it is so evident in certain patients, that I cannot conceive the slightest doubt in this regard… I had an opportunity of seeing these cells in a patient under Dr. Rayer at the La Charité Hospital. This man was affected by arteritis especially in his leg vessels. Both legs showed ecchymoses and gangrenous blisters. The blood of this patient showed such a number of white cells that I thought his blood was mixed with pus, but in the end, I was able to observe a clear-cut difference between these cells and the white cells… In fact, I believe that the excess of white blood cells is due to an arrest of maturation of blood… From my theory on the origin of blood cells, the overabundance of white blood cells should be the result of an arrest of development of intermediate cells"[16]. Here we find leukemia linked with abnormal blood pathology for the first time in medical history. He made this description of the disease in 1844, calling it an unknown disease whose findings were not published until 1855[17]. Among other published cases, two might suggest symptoms of chronic leukemia and the blood examined after death showed that the purulent matter and lymph has been mixed with the blood and had been circulated[10,18]. John Hughes Bennett (Figure 3), pathologist at the Royal Infirmary Edinburgh, gave leukemia its first published recognition as a clinical entity and as a blood-related disease. He was then often referred to as the person who first discovered leukemia because his description was more complete and scientific in nature. Bennett became interested in the disorder when his mentor, Dr. David Craigie, observed two patients admitted to the Royal Infirmary in Edinburgh with unusual blood consistency and a splenic

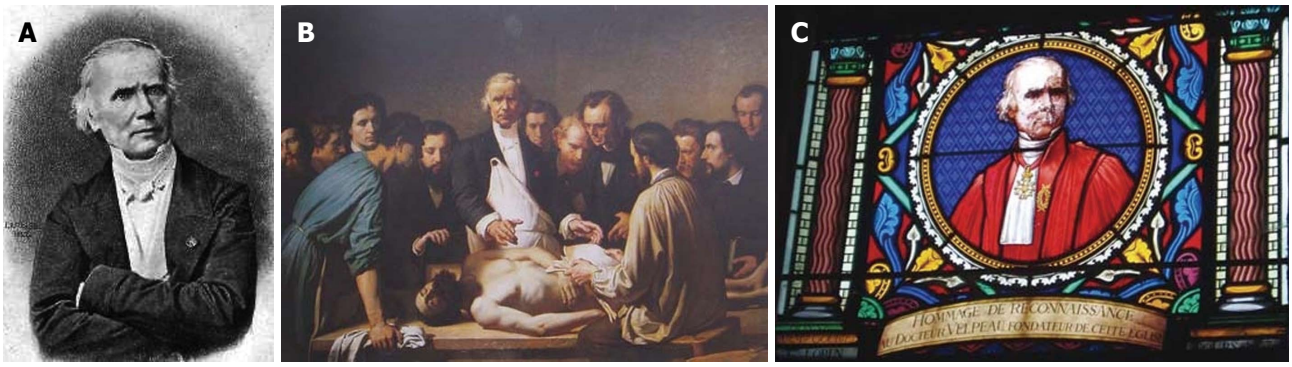

Figure 1 Alfred Velpeau (1795-1867). A: Alfred Armand Louis Marie Velpeau was born on 18 May 1795 in the Touraine village of Bréches in France, where his father was a farrier. He was expected to follow his father's footsteps, but a chance event changed his life. Interested in medicine, in an attempt to dispel the sadness of a depressed young girl, he poisoned her with hellebore. The local physician called for help was so impressed by his knowledge and intelligence that he introduced him to Vincent Gourand, surgeon at the hospital of Tours, who in turn passed him to Pierre-Fidéle Bretonneau in 1816; B: Velpeau was 21 years old[49,50]. Bretonneau was one of the outstanding physicians of his day in France. He quickly recognized the exceptional talent of his young assistant, treated him like a son, and trained him in clinical medicine and pathology. By 1819, Velpeau was "*officier de santé*" at the hospital. In 1820, Bretonneau sent him to Paris and obtained for him a position in the Saint-Louis hospital. There Velpeau gained both the anatomy and physiology prizes, while also teaching junior medical students. In 1823, Velpeau qualified and was appointed "*agrégé de medicine*" with honors, writing his thesis in Latin under the direction of Laennec on intermittent and chronic fevers, based on studies made with Bretonneau in Tours. At the age of 29 years, Velpeau came to be appointed to the junior surgical staff of various hospitals: Saint-Antoine, La Pitié, and La Charité. In 1828, he passed the "*Chirurgical*", a higher degree in surgery, and was appointed surgeon to La Pitié. Five years later, he took the university chair of clinical surgery, a position he then held for the next 33 years. Throughout his life, his work was enormous. His published works included 340 titles. There were texts on surgical anatomy, obstetrics, operative medicine, embryology, and diseases of the uterus and breast. Velpeau's "hernia", "canal", "deformity", and a "pressure bandage" for the treatment of phlebitis and burns are among the items linked with his name that have come down to us. Velpeau was elected to the Academy of Medicine in 1832 and to the prestigious Academy of Science in 1843. In 1860 honoured and famous, he visited Bréches, where he had been born. He gave a substantial sum of money to renovate the village church; C: His generosity is still remembered in a stained glass window there, in which he is represented in his professional dress with the inscription "*Hommage de reconnaissance au Docteur Velpeau, fondateur de cette église*". In 1867, Velpeau caught flu. He died on 24th of August, few days after performing an amputation. His funeral at Saint-Thomas d'Aquin and at the cemetery of Montparnasse was magnificent. It was a fitting end to the life of a man who, from humble origins, had by his own endeavours risen to the front rank of his profession as one of the leading surgeons of the century.

tumor. The first patient was observed in 1841 but was dismissed as unusual until 1844 when a 28-year-old man presented with similar symptoms. John Bennett was given permission to perform an autopsy and study the pathology of this second case. Bennett had attended the lectures on clinical microscopy given by Alfred Donné in Paris and supported Donné's ideas to use the microscope as a clinical instrument. His report entitled "Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood" was published in the "*Edinburgh Medical and Surgical Journal*" in October 1845[19]. The symptoms described together with the extensive post mortem report would today be diagnosed as chronic granulocytic leukemia. His drawings were the first illustrations of the blood cells of a patient with leukemia[20]. The second case of leukemia, published 6 wk later, was reported by Virchow[18] (Figure 4), a demonstrator in pathological anatomy at the Charité Hospital in Berlin, who described a similar case with enlargement of the spleen specifying, however, that the excess of cells was not purulent matter but instead originated in the blood. A 50-year-old woman was admitted to the Charité Hospital in Berlin complaining of fatigue, nosebleeds, swelling of the legs and abdomen, and died within 4 mo. Virchow noted the enlarged spleen and liver, but also described blood vessels filled with material resembling pus. Virchow described the disparity between white and red blood cells as "weisses blut" (white blood). Few years later, Henry Fuller, a physician at St George's Hospital in London described the first case of childhood leukemia[21]. In 1852, Bennett published the first data collection of 35 cases of leukemia or cases which might suggest leukemia. These first series demonstrated a wider geographical distribution of the disease.

CONTROVERSY ABOUT THE PRIORITY OF THE DISCOVERY

Controversy ensued from 1854 on the naming of the new disease and on the priority of its discovery: Bennett arguing precedence on the basis of the publication date and Virchow[22] suggesting that Bennett's claim was false because of his incorrect determination of purulent matter. In 1847, Virchow[22] reported a second case and for the first time used the name "leukämie" (leukemia) (the name leukemia is a combination of the Greek words "leukos" and "heima" which means "white blood") to describe this newly observed disease. This refers to the abundance of white blood cells in the body. In 1852, Bennett recommended the term "leucocythaemia", meaning increased white blood cells, which was better accepted[23]. In 1854, Kölliker[24] wrote the history of the discovery as it was understood in Germany. Bennett[23] replied in the same journal and defended his position. However, there was mutual respect between the two men. Virchow wrote testimonials in support of Bennett's candidature for two chairs in Edinburgh in 1848 and then in 1855: "I hereby testify, that having for a long time assiduously followed the very valuable researches of Dr. J Hughes Bennett of Edinburgh, I entertain the highest esteem for his scientific contributions, and consider them as among the most important of

Figure 2 Alfred François Donné (1801-1878). Alfred François Donné was born on 13 September 1801 at Noyon (France). At the age of 20 years, he moved to Paris with his family. Although he had a dislike of law as a career, he embarked on this discipline at his parents' insistence. He qualified as a lawyer but did not practice, and then became a late starter as a medical student, when he entered the Paris Faculty at the Sorbonne at 25 years of age. A few years later he married Marie Antoinette Joantho, and through his wife, became linked with a well known medical family, the des Essarts. Donné graduated in 1831 at the age of 30 years. His clinical work was concentrated at the Charité hospital under the very experienced clinician Bouillaud. His qualities were recognized by the University Council and they appointed him to the honorary post of sub-librarian to the Faculty of Medicine[51]. In 1836, he made one of his greatest contributions to medicine by discovering the protozoon, *Trichomonas vaginalis*, in vaginal secretion of Parisien prostitutes[52] and recorded it in a publication addressed to the Academy of Sciences. During this period of intensive research and clinical work, Donné had realized that microscopes were invaluable for the proper illustration and understanding of his lectures. His pediatric successes with feeding difficulties of premature infants led to his election as a Chevalier of the Legion of Honor and his nomination as Inspector General of Medicine. His successful researches into hematology have not received the publicity and fame they deserve. In1842, he announced his discovery of blood platelets to the Academy of Sciences and these were incorporated in his *"Atlas de microscopie médicale"*[53]. After loosing his office of Inspector General following the 1848 revolution, he was installed in 1853 as the new Rector of the University of Strasbourg, then became in 1855 Rector of the University of Montpellier. During his stay of almost 20 years at Montpellier, he became interested in theories and studies on spontaneous generation. On retiring from office in 1875, he returned to Paris where he died of a cerebral vascular accident on 7 March 1878[51]. He remains virtually unknown outside of France, never obtained the title of professor and was a practical man fond of microscope and laboratory work, but his contribution to medical and scientific progress is inestimable.

those which have enriched the Pathology now struggling for a physiological basis. It appears to me, therefore, that it would be only a well-merited reward, if the Chair of Physiology and Pathology should be given to him, who is able to fill it so worthily". The question of priority was resolved publicly by Virchow in a lecture he delivered in 1858, in which he stated that Bennett observed a case of indubitable leukemia few months before he saw his first case: "It is moreover the same conclusion which Bennett came to in the much discussed matter of priority between us when he observed a case of individual some months before I saw my first case".

IDENTIFICATION OF LEUKEMIA AS A SEPARATE DISEASE

Pus and inflammation continued to dominate hematological thoughts until the middle of the nineteenth century. In a third publication (1856), Virchow[25] was credited with concluding that the disorder was not the result of an in-

Figure 3 John Hughes Bennett (1812-1875). Born in London on 31 August 1812, Bennett (Figure 3) was educated at Exeter (England) and being destined for the medical profession, he entered an apprenticeship with a surgeon in Maidstone (Kent). In 1833, he began his studies in Edinburgh. He published his first article in "*London Medical Gazette*" in 1836. He graduated in 1837 with the highest honors and gold medal, with a dissertation entitled "The physiology and pathology of the brain". During the next 4 years, he studied in Paris, where he founded the English-speaking Medical Society, and then in Germany. On his return to Edinburgh in 1841, he published a "Treatise on cod-liver oil as a therapeutic agent" and became physician at the Royal Public Dispensary of Edinburgh. He began to lecture as an extra-academic teacher on histology, drawing attention to the importance of the microscope in the investigation of diseases[54]. In 1843, he was appointed professor of the Institute of Medicine in Edinburgh. Opposed bloodletting and the indiscriminate use of drugs, he was an important influence in changing British therapeutic practices during the second half of the nineteenth century. In 1845, he published a paper entitled "Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood" in the "*Edinburgh Medical and Surgical Journal*". In 1846, he became editor and later proprietor of the Monthly Journal of Medical Science. In 1851, Bennett founded and became the first president of the Physiological Society of Edinburgh. His publications were very numerous including "*Lectures on clinical medicine*" (1850-1856), "*Clinical lectures on the principles and practice of medicine*", "*Leucocythaemia*" (1852), "*Outlines of physiology*" (1858), "*Pathology and treatment of pulmonary tuberculosis*" (1853), "*Textbook of physiology*" (1871-1872). In 1869, he supported the admission of women medical students in Edinburgh. In 1873, he was elected a member of the French Academy of Medicine and granted recognition by the French government to practice medicine in France. In 1875, after his participation at the meeting of the British Medical Association, he was compelled to have the operation of lithotomy performed. He sank rapidly and died on September 25 at Norwich. In 1901, the University of Edinburgh inaugurated the John Hughes Bennett Laboratory of Experimental Pathology. A second laboratory with his name was opened in 1998, in a joint venture between Britain's Leukaemia Research Fund, the University of Edinburgh and the Western General Hospital Trust.

fectious process but rather was caused by the tissue that produced the white blood cells. The debate centred upon whether leukemia was in fact a separate disease was then conducted by some of the leading physicians in France. Among the minority that supported the autonomy of leukemia, Gabriel Andral, Professor at the University of Paris (Figure 5), proposed the study of the blood as a clinical discipline and defended the use of the microscope in clinical medicine. He was the founder of the science of hematology and is credited with the integration of that science into clinical and investigative medicine. Leukemia gradually became accepted as a distinct disease and clinical and pathological description became more detailed. However, the definition of leukemia was far from precise until a first classification was introduced in 1857 by Nikolaus Friedreich, a pathologist in Würzburg (Figure 6).

Figure 4 Rudolph Virchow (1821-1902). Born in Germany in 1821, he studied medicine and chemistry in Berlin at the Prussian Military Academy from 1839 to 1843. After graduation in 1843, he went to serve as assistant at the Charité Hospital. In 1847, he qualified as a lecturer at the University of Berlin and participated in founding the "Archiv für pathologische anatomie und physiologie and für klinische medizin". Virchow is credited with multiple important discoveries. Besides his role in recognizing leukemic cells, he was one of the first to accept the work of Robert Remak who showed that the origin of cells was the division of preexisting cells. He also described that an enlarged left supra-clavicular node is one of the earliest signs of gastrointestinal malignancy. He elucidated the mechanism of pulmonary thromboembolism and founded the medical fields of cellular pathology and comparative pathology. He also developed a standard method of autopsy procedure. In 1861, he was elected a foreign member of the Royal Swedish Academy of Sciences. In 1862, he was awarded the Copley Medal. In 1869 Virchow founded the Society of anthropology, ethnology and prehistory which was very influential in coordinating German archaeological research[55,56]. More than a laboratory physician, Virchow was an impassioned advocate for social and political reform. He made himself known as a pronounced democrat in the year of revolution, 1848, and his political activity caused the government to remove him from his position. In 1859, he became a member of the Municipal Council of Berlin, and began his career as a civic reformer. Elected to the Prussian Diet in 1862, he became leader of the Radical or Progressive party, and from 1880 to 1893 he was a member of the Reichstag. He is widely regarded as a pioneer of "social medicine", focusing on the fact that disease is never purely biological, but often socially derived or spread[57]. Virchow died of heart failure in 1902.

Figure 5 Gabriel Andral (1797-1876). Gabriel Andral was born in Paris, the son of a well-known physician who was a member of the academy and personal physician to the French revolutionary leader Jean-Paul Marat. Andral received his doctorate in 1821 with a thesis on expectoration. He was habilitated in 1824 and became "*agrégé*". The faculty appointed him professor of hygiene in 1828 on the death of René-Joseph-Hyacinthe Bertin. When baron René-Nicolas-Dufriche Desgenettes retired, he became professor of internal pathology, and in 1839 he succeeded François-Joseph-Victor Broussais in the chair of general pathology and therapy, holding this tenure for 27 years. Besides he was physician at the Charité. In 1823, Andral became a member of the Academy of Medicine. In 1843 he became member of the Institute, and in 1858 was made a commander of the Legion of Honor. In 1866 he abandoned his chair and retired, but still he took part in the advance of science and participated in the transactions of learned societies. He died of a heart condition on February 13, 1873. His main oeuvre "*Clinique médicale*", a five-volume work, comprises almost every aspect of medicine. This treatise on general medicine may be considered a summary of French medicine as it had developed in the first decades of the 19th century. He was the founder of the science of hematology. He is said to be the originator of the word "anemia" and was the first physician to see the potential of chemical analysis of the blood.

THE MORPHOLOGICAL ERA OF HEMATOLOGY

During his period of intensive research and clinical work, Donné had realized that microscopes were invaluable for the proper illustration and understanding of his lectures. In spite of hostility, he organized a microscopy course (the first microscopic workshop in medicine) that attracted French as well as foreign students. One of Donné's foreign students was John Hughes Bennett. After Louis Daguerre, one of the inventors of photography presented his discovery to the Academy of Sciences, Donné become most interested in these photographic reproductions and resolved to incorporate them in his lectures. In 1844 he described his procedure in a book entitled "*Cours de microscopie complémentaire des études médicales*" which demonstrated the scope of the instrument[16]. The first reported use of the microscope to diagnose leukemia in a living patient was performed by Fuller[26]. The neoplastic nature of leukemia and the origin of the disease in organs of blood formation having been established, the clinical and biological science of hematology were given a tremendous boost in the period between 1878 and 1888, when it became possible to examine the microscopic details of

Figure 6 Nikolaus Friedreich (1825-1882). Born in Würzburg in 1825, Nikolaus Friedreich received medical training in this city where his father and grandfather had been professor of medicine. He received his doctorate in 1850. He became Assistant at the clinic of clinician Karl Friedrich von Marcus and in 1853 was habilitated as Privatdocent of special pathology and therapy. When Virchow came to Würzburg, Friedreich became an ardent student of this great pathologist and considered abandoning clinical medicine for pathology. In 1857, he was appointed professor of pathological anatomy at Würzburg, and in 1858 moved to the tenure of professor ordinarius of pathology and therapy at Heidelberg, a post which he held for the remainder of his career. He was also director of medical clinic. He took an interest in all branches of medicine, especially neurology. He has left 8 major and 51 larger and smaller treatises, among them a number of monographs. These include works on leukemia. He died in 1882 from a ruptured aortic aneuvrysm.

Figure 7 Paul Ehrlich (1854-1915). Paul Ehrlich was born on March 14, 1854 at Strehlen in Silesia (Germany). Educated at the Gymnasium at Breslau and subsequently at the universities of Breslau, Strassburg, Freiburg-im-Breisgau and Leipzig, he obtained his doctorate of medicine in 1878 by means of a dissertation on the theory and practice of staining animal tissues. In 1882 he published his method of staining the tubercle bacillus that Koch had discovered. This method was the basis of the subsequent modifications introduced by Zhiel and Neelson and of the Gram method of staining bacteria. In 1882 Ehrlich became Titular Professor and in 1887 he qualified as a Privatdozent in the faculty of medicine in the University of Berlin. Later he became an Associate Professor and Senior House Physician to the Charité Hospital in Berlin. After becoming Robert Koch's assistant in 1890, he began immunological studies. He worked out the details of preparing an antitoxin for diphteria, which represented the first use of immunotherapy to specifically treat an infection. In 1896 he was appointed Director of the Institute for the control of therapeutic sera at Steglitz in Berlin, and formulates his side-chain theory of immunity. In 1897 Ehrlich was appointed Public Health Officer at Frankfurt-am-Main and in 1899 became director at the Royal Institute of Experimental Therapy. He then began another phase in his varied researches and devoted to chemotherapy with the aim to find chemical substances which have special affinities for pathogenic organisms and would be "magic bullets" which would go straight to the organisms at which they were aimed. He produced trypan red and established the correct structural formula of atoxyl effective against tryptosomes. This open the way of obtaining new organic compounds with trivalent arsenic. One arsenic drug was found very effective against syphilis. Ehrlich announced it under the name of "Salvarsan". Another arsenical substance named "Neosalvarsan" became more easily administered. During the later years of his life, Ehrlich was concerned with experimental work on tumors and on his view that sarcoma may develop from carcinoma, also on his theory of athreptic immunity of cancer. In 1908 he shared with Metchnikoff the highest scientific distinction, the Nobel price. On August 20, 1915 a stroke ended his life.

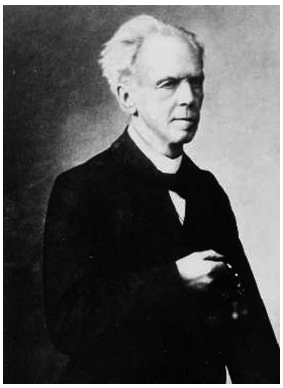

Figure 8 Ernst Neumann (1834-1918). Ernst Neumann was born in 1834 at Königsberg, capital of eastern Prussia, as great son of Karl Goofried Hagen (professor of chemistry and pharmacy) and son of Franz Ernst Neumann (a pioneer in the mathematical physics). In 1850, he enrolled at the famous university of Königsberg (Albertina). In 1855, he took his doctors advice degree. After studies in Prague, Berlin (under Rudolf Virchow) and Königsberg, he became a lecturer in medicine in 1859. He got interested in the rising field of pathological anatomie and was appointed professor of pathology at Königsberg in 1866. He described the presence of nucleated red blood cells in bone marrow sap of humans and rabbits obtained by squeezing bones. He was the first to conclude that during postembryonic life, erythropoiesis is taking place in bone marrow. Further studies pointed to the fact that leukocytes are also formed in the bone marrow. He postulated a common stem cell for all hematopoietic cells. He later was made Geheimer Medicinalrath - privy medical councellsor, and received honorary doctorate from the universities of Tübingen and Geneva, in 1898 and 1915. He died in 1918.

blood cells. The event that provided this opportunity was biological stains. At this point of medical history, despite the fact that iodine, suffron, and ammonia carmine were available for staining tissues and cells, practically no advances were made in morphology of blood cells. The state of hematology from the 1850s to the 1870s was delineated by Beale[27] (1828-1906), a British professor, in a book that went through several editions. Beale[27] illustrated and described "the various corpuscules met within healthy blood". A major advance in understanding leukemia pathophysiology came in 1877 when Ehrlich[28] (Figure 7) developed a triacid stain and introduced the names acidophil (later changed to eosinophil), basophil and neutrophil for the three different granulocyte types. His techniques initiated the true morphological era of hematology. In 1891, the triacid stain was replaced by the eosine methylene blue stain invented by DL Romanowsky (1861-1921) of St. Petersburg in Russia. The "Romanowsky stain" was further modified by Richard May of Munich in 1902, Gustav Giemsa (1867-1948) of Hamburg in 1905, and JH Wright of Boston in 1906. However, all of these modifications were direct descendants of Ehrlich's original ideas.

THE ORIGIN OF LEUKEMIA

A vital discovery came in 1868 when Neumann[29] (Figure 8), professor of Pathological Anatomy at Königsberg, reported changes in the bone marrow in leukemia and established the link between the source of blood and the bone marrow. One year later, he published an extensive description of cells in the marrow, and, 1 year later again, that the circulating red cells were derived from an ancestral cell[30]. Around the same time, Bizzozero[31,32] (1846-1901) of Pavia confirmed the observation that non-nucleated red blood cells were formed from nucleated red cells in the bone marrow and that the blood formation function of the bone marrow also included white blood cells. He also identified the platelet[33]. Neumann[34] stated in 1872 that leukemia was a disease of the marrow. Shortly thereafter, Mosler[35] introduced bone marrow puncture as a means of antemortem diagnosis of leukemia. For some years, the mystery remained of how the cells were able to travel through the bone to the circulatory blood system.

THE CONCEPT OF THE "STEM CELLS"

The concept of "stem cells" was first proposed by German zoologists and medical scientists[36]. Adopting the term "*stammzelle*" from Ernst Haeckel (1834-1919), a con-

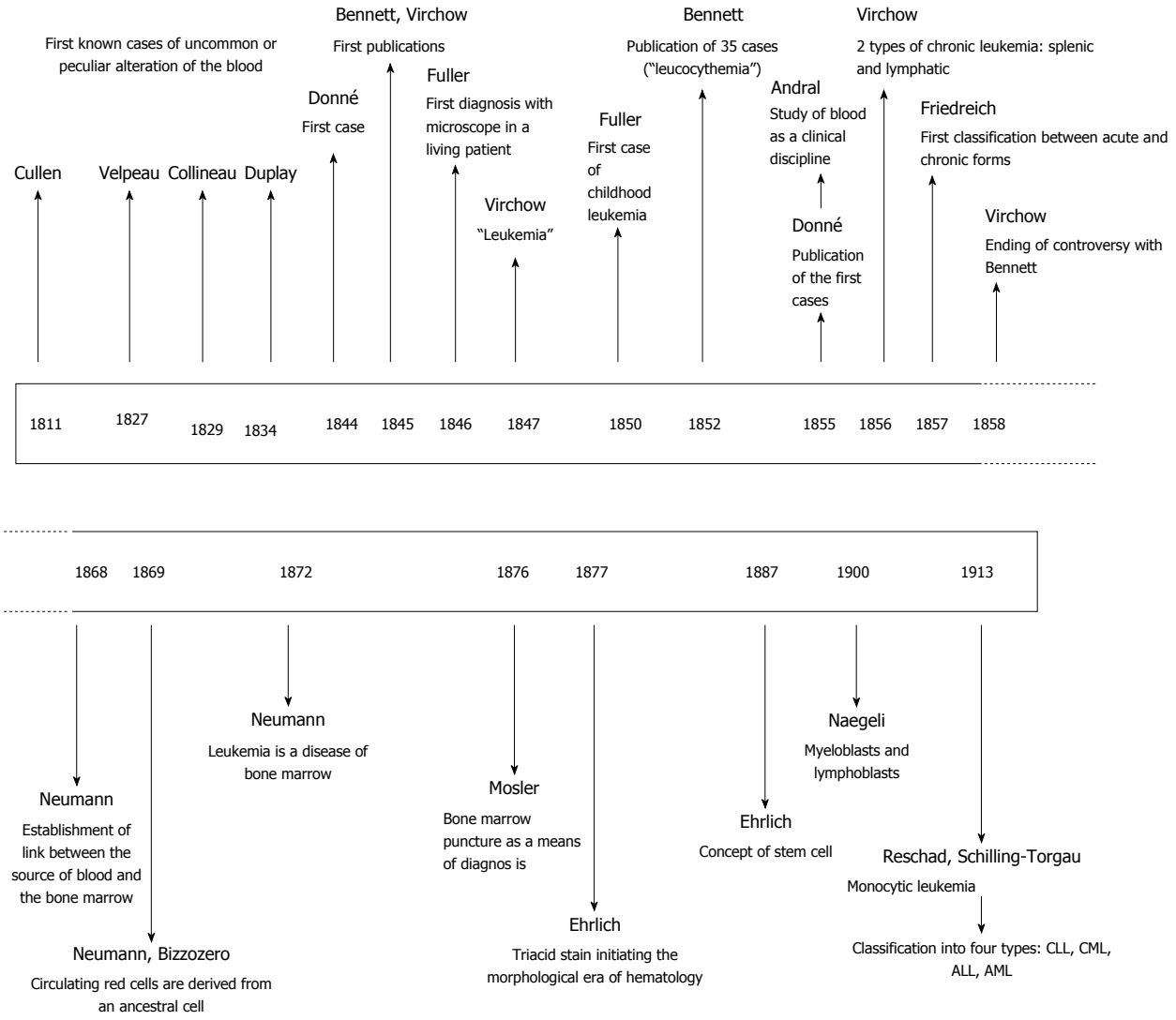

Figure 9 First contributors in the history of leukemia. CLL: Chronic lymphocytic leukemia; CML: Chronic myeloid leukemia; ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia.

troversial Darwinist and professor of zoology at Jena[37], Theodor Boveri (1862-1915) was influential in making these cells known as carriers of the so-called "germ plasm" and as the starting points in embryological development of differentiated body cells as well as germ cells[36]. The essential characteristic of a "stem cell" was a capacity for self-renewal as well as for differentiation into specific types of somatic cells or germ cells. Valentin Haecker (1864-1915), who was made director of the Zoological Institute at the University of Stuttgart and subsequently at the University of Halle, propagated the notion of "pluripotency", which he ascribed to the "germ plasm" of an organism[38]. In 1896, Artur Pappenheim (1870-1916), who worked at Virchow's Pathological Institute in Berlin on the formation of red blood cells, developed the conception that "stem cells" or "mother cells" ("mutterzellen") (as he called them) were embryonic cells that had the potential to differentiate into different cell lines and to form the basis of different types of blood cells[39]. Papenheim also argued that myelocytes and lymphocytes originated from the same "multipotent stem cell"[40]. Julius Cohnheim's theory was also widely discussed at the end of the nineteenth-century. He defined tumors as "atypical neoplasms of tissue based on an embryonic rudiment"[41]. The lymphocyte was described as the "common, indifferent stem cell" for erythrocytes and granulated granocytes by Dantschakoff[42] or even as the "common stem cell" of all types of blood cells by Maximow[43]. These authors were committed to the "Unitarian" view of the different types of blood cells. In contrast, Ehrlich[44] was the first scholar to propose the "dualist doctrine", which assimed that the lymphocytes and leucocytes originated from morphologically different precursor cells in different organs: lymph nodes and spleen for the first ones and bone marrow for the second ones. In 1900, the Swiss hematologist Naegeli described a new cell in the myeloid cell line, which he named the myeloblast, as an ancestor of granulocyte cells[45]. He also showed the lymphoblast as an ancestor of lymphocytes. The presence of myeloblasts or lymphoblasts in the circulating blood formed a classic diagnosis of acute leukemia. Monocytic leukemia was first described by Reschad *et al*[46], and leukemic cells recognized as unusual forms of myeloblasts.

CLASSIFICATION OF LEUKEMIAS

In 1856, Virchow[25] categorized two types of the disease - the splenic and the lymphatic forms - by the starting site of the disease, which we now know as leukemia and lymphoma, respectively. A first classification was introduced in 1857 by Nikolaus Friedreich. A distinction between acute and chronic forms of leukemia was appreciated. He reported at length on a case which he described as acute leukemia, the first time the term was used[47]. Few years later, Mosler added the classification myelogenous leukemia to the splenic and lymphatic types of the disease[48]. The development of a triacid stain by Paul Ehrlich in 1877 simplified the classification of leukemia into the myeloid group and the lymphoid group. Towards the end of the nineteenth century, a clearer definition of the classification of the subtypes of leukemia had been established, but there was still a lack of any form of effective therapy. This is only in 1913 that leukemia was classified into four types: chronic lymphocytic leukemia, chronic myelogenous leukemia, acute lymphocytic leukemia, and acute myelogenous leukemia.

CONCLUSION

While the modern era of leukemia chemotherapy began recently, the recognition of leukemias has been mainly recorded in the second part of the nineteenth century. This brief historic review reports the first descriptions of the disease and the major advances in its history from its roots to the beginning of the twentieth century. Although most treatments for leukemia were ineffective until the middle of the twentieth century, it seemed of interest to review some pertinent exemples of the evolution in the knowledge of this disease (relied upon chronology as an organizing framework, while stressing the importance of themes), since our current knowledge about leukemia is still mainly based on the first accounts of scientific and medical discovery. Towards the end of the nineteenth century, a clearer definition of the classification of leukemia had been established leading to different subtypes. These well-defined subtypes of leukemia were used for the development of effective chemotherapy, which has represented the most important advance in leukemia research during the past half century (Figure 9).

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REVIEW

PVSG and WHO *vs* European Clinical, Molecular and Pathological Criteria for prefibrotic myeloproliferative neoplasms

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Abstract

The Polycythemia Vera Study Group (PVSG), World Health Organization (WHO) and European Clinical, Molecular and Pathological (ECMP) classifications agree upon the diagnostic criteria for polycythemia vera (PV) and advanced primary myelofibrosis (MF). Essential thrombocythemia (ET) according to PVSG and 2007/2008 WHO criteria comprises three variants of JAK2^{V617F} mutated ET when the ECMP criteria are applied. These include normocellular ET, hypercellular ET with features of early PV (prodromal PV), and hypercellular ET due to megakaryocytic, granulocytic myeloproliferation (ET.MGM). Evolution of prodromal PV into overt PV is common. Development of MF is rare in normocellular ET (WHO-ET) but rather common in hypercellular ET.MGM. The JAK2^{V617F} mutation burden in heterozygous mutated normocellular ET and in heterozygous/homozygous or homozygous mutated PV and ET.MGM is of major prognostic significance. JAK2/MPL wild type ET associated with prefibrotic primary megakaryocytic and granulocytic myeloproliferation (PMGM) is characterized by densely clustered immature dysmorphic megakaryocytes with bulky (bulbous) hyperchromatic nuclei, which are never seen in JAK2^{V617F} mutated ET, and PV and also not in MPL^{515} mutated normocellular ET (WHO-ET). JAK2^{V617} mutation burden, spleen size, LDH, circulating CD34^{+} cells, and pre-treatment bone marrow histopathology are mandatory to stage the myeloproliferative neoplasms ET, PV, PMGM for proper prognosis assessment and therapeutic implications. MF itself is not a disease because reticulin fibrosis and reticulin/collagen fibrosis are secondary responses of activated polyclonal fibroblasts to cytokines released from the clonal myeloproliferative granulocytic and megakaryocytic progenitor cells in ET.MGM, PV and PMGM.

Key words: Myeloproliferative neoplasms; Essential thrombocythemia; Prodromal polycythemia vera; Polycythemia vera; Myelofibrosis; JAK2^{V617F} mutation; JAK2 wild type myeloproliferative neoplasm; Bone marrow pathology

Core tip: The integrated World Health Organization (WHO) and European Clinical, Molecular and Pathological classification of the myeloproliferative neoplasms include JAK2^{V617F} mutated normocellular essential thrombocythemia (WHO-ET), prodromal polycythemia vera (PV), classical PV, and hypercellular ET due to megakaryocytic, granulocytic myeloproliferation. Evolution of prodromal PV into overt PV is common. JAK2/MPL wild hypercellular ET associated with prefibrotic primary megakaryocytic

and granulocytic myeloproliferation is characterized by densely clustered immature dysmorphic megakaryocytes with bulky (bulbous) hyperchromatic nuclei, which are never seen in JAK2^{V617F} mutated ET and PV, and also not in JAK2 wild type normocellular ET (WHO-ET) carrying the MPL^{515} mutation.

Michiels JJ, Berneman Z, Schroyens W, Lam KH, De Raeve H. PVSG and WHO *vs* European Clinical, Molecular and Pathological Criteria for prefibrotic myeloproliferative neoplasms. *World J Hematol* 2013; 2(3): 71-88 Available from: URL: http://www.wjgnet.com/2218-6204/full/v2/i3/71.htm DOI: http://dx.doi.org/10.5315/wjh.v2.i3.71

INTRODUCTION

Vaquez[1] and Osler[2] first described polycythemia vera (PV) as a distinct disease entity. In 1950, Dameshek[3] described PV as a trilinear myeloproliferation of the bone marrow with various degrees excessive production of red blood cells, granulocytes and platelets. Dameshek[3] proposed two highly speculative possibilities for the etiology of trilinear PV: first, the presence of excessive bone marrow stimulation by an unknown factor, second, a lack or a diminution in the normal inhibitory factor. This hypothesis is confirmed by the discovery of the JAK2^{V617F} mutation by James *et al*[4] in 2005 demonstrating that the JAK2^{V617F} mutation induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2. The JAK2^{V617F} mutation makes the mutated hematopoietic stem cells hypersensitive to hematopoietic growth factors TPO, EPO, IGF1, SCF and GCSF, resulting in trilinear myeloproliferation with clinical manifestations of essential thrombocythemia (ET), PV and myelofibrosis (MF) (Figure 1)[4,5].

The Polycythemia Vera Study Group (PVSG) followed the 1951 recommendations of Dameshek to define PV by increased red cell mass (RCM) as a major criterion and proposed criteria for the clinical diagnosis of Ph-negative ET, PV and agnogenic myeloid metaplasia (AMM) with MF[6-10]. The unifying concept of the lumping of the chronic myeloproliferative disorders (MPD) ET, PV, AMM, chronic myeloid leukemia (CML) by Dameshek in 1951 has been broken up by the PVSG in 1975 into Ph-positive (Ph$^+$) CML and Ph-negative ET, PV and AMM (Figure 1)[6-11]. The Ph-negative MPDs ET, PV and MF form a benign group of chronic MPD, whereas the Ph$^+$ chromosome is the result of the *BRC/ABL* fusion gene and protein. *BCR/ABL*-positive CML appears to be a real neoplasia (leukemia) with an inevitable transition into acute leukemia when strict morphological, biochemical, cytogenetic and molecular criteria are used in routine daily practice[12-14]. The Thrombocythemia Vera Study Group introduced bone marrow biopsy as a specific, pathognomonic clue for early stage ET and PV[15,16]. The PVSG[6-8] and the 2001 World Health Organization (WHO) criteria for the classification of the MPDs are not refined enough to also take the early prefibrotic stages of thrombocythemia in various MPDs into account[15-20]. The availability of the current clinical and molecular markers endogenous erythroid colony (EEC) formation, serum EPO levels, the JAK2^{V617F} mutation and bone marrow histology allow the detection of early stage ET and PV. Within the context of the European Working Group on MPD Michiels *et al*[15-17] contributed significantly to the European consensus criteria for ET, PV and chronic idiopathic MF (CIMF) by including bone marrow histology and subsequently defined between 2002 and 2005 the European Clinical and Pathologic (ECP) criteria (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf)[17-19]. In the present study, we extend the PVSG, the ECP[18-20], and the 2007/2008 WHO[21,22] MPD/myeloproliferative neoplasms (MPN) classifications into simpified and integrated WHO and European Clinical, Molecular and Pathological (WHO-ECMP) criteria by including bone marrow pathology together with a complete set of established laboratory and molecular markers for diagnostic differentiation of each of the latent (masked), early and overt MPNs[19,20].

DIAGNOSIS OF MPN

ET

In the 1980s, Georgii *et al*[23-25] and Thiele *et al*[26-29] defined the pathological features of ET, PV and chronic megakaryocytic granulocytic myeloproliferation (CMGM) or CIMF as derived from bone marrow histopathological morphology (Figure 1)[30]. ET was defined by Georgii *et al*[23-25] and Thiele *et al*[26-29] as persistent increase of platelets in excess of 400 × 10^9/L without the Ph$^+$ chromosome together with monolinear proliferation of mature enlarged megakaryocytes in the bone marrow with normal cellularity, normal erythropoiesis and normal granulopoiesis (Figure 1). This bone marrow definition for the diagnosis of normocellular ET has been used by Michiels *et al*[17,18] in the ECP classification of MPD (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and by the 2007/2008 WHO classification (WHO-ET)[21,22]. Normocellular ET (WHO-ET) only comprises about one third of PVSG defined ET patients[21,22]. The 1997 PVSG and 2001 WHO classifications used a platelet count in excess of 600 × 10^9/L as the minimum criterion for the diagnosis of ET[7,14] and therefore did not include the early stages of ET, which consequently were diagnosed as masked or unclassifiable MPD[18]. This comprises about 30% of early stage or latent ET (Table 1) indicating the need to lower the platelet count cut-off to 400 × 10^9/L (upper limit of normal) for the diagnosis of thrombocythemia in various MPDs (Table 2)[12-20]. The relatively high incidence of early prefibrotic thrombocythemia with a platelet count between 400 and 600 × 10^9/L strengthen the need for use of specific laboratory and molecular markers to differentiate thrombocythemia from reactive thrombocytosis followed by bone marrow histopathological evaluation in order to correctly diagnose patients with suspected

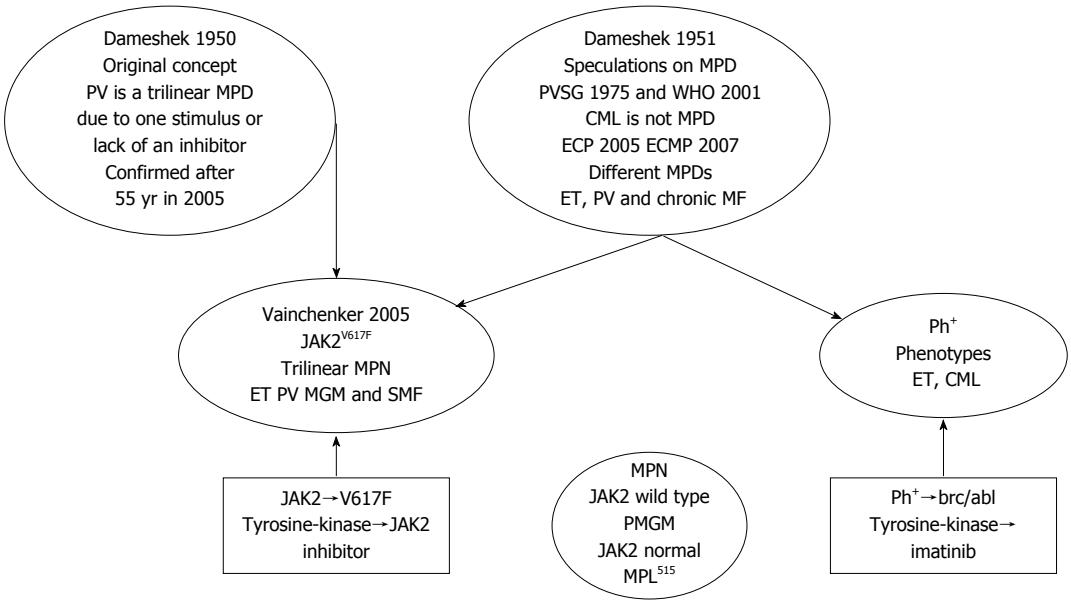

Figure 1 The concept of Dameshek in 1950 on polycythemia vera as a trilinear myeloproliferative disorder due to an unknown excessive bone marrow stimulating factor and/or a lack or a diminution in the normal inhibitory factor, which appeared to be caused by the acquired heterozygous and/or homozygous JAK2^{V617F} mutation discovered by James *et al*[5]. The unifying concept of Dameshek in 1951 on lumping the chronic disorders [myeloproliferative disorder (MPDs)] essential thrombocythemia (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (AMM) has been broken up by the Polycythemia Vera Study Group (PVSG) in 1975 into Ph-positive (Ph$^+$) thrombocythemia and chronic myeloid leukemia (CML) and the Ph-negative MPDs ET, PV and myelofibrosis (MF). In 2005, PV indeed proved to be a JAK2^{V617F} mutated trilinear MPD, whereas ET and PMF are either positive or negative for the JAK2^{V617F} mutation. PMGM: Primary megakaryocytic and granulocytic myeloproliferation; MPN: Myeloproliferative neoplasm; WHO: World Health Organization; ECP: European Clinical and Pathologic; ECMP: European Clinical, Molecular and Pathological; MGM: Megakaryocytic, granulocytic myeloproliferation.

MPN[18-20]. The 2007/2008 WHO classification reduced the platelet count from 600 × 10^9/L to 450 × 10^9/L and added bone marrow features as major criteria for normocellular ET (WHO-ET, but did not define criteria for hypercellular ET, Table 2)[21,22]. Recent studies clearly show that PVSG defined ET according to ECMP criteria include at least three phenotypes of ET at the bone marrow level (Tables 2 and 3, Figure 2)[19,20]. About 50% of PVSG defined ET patients show not only spontaneous EEC but also increased score for leukocyte alkaline phosphatase (LAP) together with low serum EPO levels (Table 2)[31-36] indicating that EEC-positive ET with low serum EPO comprises a biologically distinct subgroup of ET patients reflecting early PV ("forme fruste" PV, Table 2) that is at risk for progression to overt PV (Table 3). Spontaneous EEC formation is the hallmark of PV. In a study of 170 PVSG-defined ET patients, spontaneous EEC formation was seen in all 11 (6.5%), who later developed PV, but also in 60% of 159 patients with stable ET during a median follow-up of 29 mo (12-138 mo)[37]. This overlap of EEC in ET and PV points to the need for specific molecular and pathological markers to better differentiate between normocellular ET and hypercellular ET from prodromal PV and classical PV (Table 2, Figures 2-5)[38,39].

PV

In the 1980s bone marrow, Georgii *et al*[23-25] and Thiele *et al*[26-29] used a typical trilinear hypercellular bone marrow with increased megakaryopoiesis, erythropoiesis and granulopoiesis (panmyelosis) as mandatory criteria for the diagnosis for classic PV (Figure 2). This definition is used by the ECP (http://www.mpn-stichting.nl/doctors_brochure_2004.pdf) and the 2007/2008 WHO classification to confirm the diagnosis of PVSG defined PV in cases with increased RCM or increased hemoglobin and hematocrit above the upper level of normal and in cases with JAK2^{V617F} mutated erythrocythemia[15-22]. The PVSG criteria use increased RCM or persistent high levels for hemoglobin and hematocrit as a major crude inclusion criterion and a histological bone marrow picture characteristic for PV as a minor criterion for the diagnosis of PV, thereby excluding early thrombocythemic stage PV mimicking ET (Table 3, Figure 2). Spontaneous EEC formation and low serum EPO levels are used as specific criteria for the diagnosis of PV, but have insufficient diagnostic sensitivity as isolated parameters to differentiate between PV, congenital polycythemia (CP), secondary erythrocytosis (SE), ET and normal controls[31-34]. RCM measurement is still the WHO gold standard to distinguish ET from PV and to distinguish idiopathic from apparent erythrocytosis. In patients with so-called idiopathic erythrocytosis (increased RCM, hemoglobin and hematocrit but normal leukocyte and platelet counts and no splenomegaly), the histological evaluation of the bone marrow clearly differentiate between erythrocythemic early stage PV showing increased erythropoiesis and loosely clustered large pleomorphic megakaryocytes from CP and SE with increased erythropoiesis and megakaryocytes of normal size and morphology[15-19]. Increased RCM alone does not distinguish early erythrocythemic PV from CP or SE, indicating the need of specific clinical, molecular markers including JAK2^{V617F} and MPL^{515} mutations and bone marrow histology. In patients with JAK2^{V617F} mutated ET,

Table 1 Blood and bone marrow features in one prospective study of Thrombocythemia Vera Study Group-defined essential thrombocythemia and one retrospective study of Polycythemia Vera Study Group defined essential thrombocythemia at platelet counts above the upper limit of normal

Ref.	Michiels *et al*[11]	Lengfelder *et al*[30]	
Type of study	Prospective 1975-1985	Retrospective 1975-1995	
Diagnosis ET	TVSG criteria	PVSG criteria	
Inclusion criterion	ET	ET	Tentative diagnosis
Platelet count × 10^9/L	> 400	> 350	WHO-ECMP
Number of ET patients	30	143	
Platelets × 10^9/L range	420-1500	< 350-> 000	
Below 600	13%	29%	Early latent ET
Between 600-1000	54%	45%	Fits with ET
Above 1000	33%	26%	Fits with ET
Leukocytes			
Above 12 × 10^9/L	10%	51%	
Hemoglobin			
Below 16 g/dL	-	80%	
Below 17 g/dL	-	100%	
Above 16 g/dL	-	20%	Fits with PV
Splenomegaly			
No	63%	56%	
Yes	37%	44%	
Spleen size on echogram (cm)			
n < 12/12-15/> 15	2019/8/3	-	
Bone marrow biopsy			
Normal cellularity	17 (57%)	52%	Fits with true ET
Increased cellularity	13 (43%)	60%	
Increased erythropiesis	13 (43%)	17%	Fits with early PV
Increased granulopiesis	0	45%	Fits with CMGM
Myelofibrosis	No	No	

Essential thrombocythemia (ET) according to Polycythemia Vera Study Group (PVSG) criteria appears to be a spectrum of normocellular ET, prodromal polycythemia vera (PV) and ET due to megakaryocytic, granulocytic myeloproliferation or ET associated with chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) when diagnostic World Health Organization and European Clinical, Molecular and Pathological (WHO-ECMP) bone marrow features are applied. TVSG: Thrombocythemia Vera Study Group.

slight splenomegaly and borderline erythrocytosis RCM is the gold standard to make the distinction between hypercellular ET due to increased erythropoiesis with normal RCM (prodromal PV) from classical PV with increased RCM. In our experience, cases of prodromal PV, masked PV do show a typical PV picture in the bone marrow histopathology (Figure 3). This controversial topic has been addressed in a separate report (manuscript in press). PV patients do have erythrocytes above 6 × 10^{12}/L even when the hemoglobin and hematocrit are in the upper range of normal due to microcytosis of erythrocytes caused by iron deficiency and/or significant splenomegaly[3,39]. Consequently, RCM measurement is of debatable additional diagnostic value in classic PV carrying the JAK2^{V617F} or exon 12 mutation, since all patients with 2008 WHO/ECMP defined PV do have erythrocyte counts above 6 × 10^{12}/L and demonstrate a bone marrow histology that is pathognomonic for PV (Table 3).

Nomenclature, clinical and bone marrow diagnosis of primary MF

The terms AMM and IMF are applied to hypercellular advanced fibrotic stages of MPN[8,23-29]. MF is a reactive feature secondary to progressive disease seen in AMM, CIMF, PV and CML. In 1988, 1996 and 1999 Thiele *et al*[26-29] clearly defined the bone marrow features of normocellular true ET (WHO-ET, Table 2, Figure 2), of hypercellular trilinear PV (Table 3, Figure 2), and prefibrotic CIMF with associated thrombocytosis (Figure 2). According to Thiele *et al*[26-29], "true" ET clearly differs from ET associated with prefibrotic CIMF labeled as "false" ET. In true ET megakaryocytes display large to giant megakaryocytes showing hyperlobulated staghorn-like nuclei in a normocellular bone marrow (WHO-ET, Table 2, Figure 2). Interestingly, the megakaryocytes in true ET are larger than in PV[26]. PV is typically featured by small to large (pleomorphic) megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased erythro-granulocytic myeloproliferation (WHO-PV, Table 3, Figures 2, 3 and 5). In 1980, Georgii *et al*[23] described CMGM as a distinct MPD entity apart from ET. In 1990, Georgii *et al*[24,25] proposed the Hannover Bone Classification of the myeloproliferative disease and defined CMGM as hypercellular prefibrotic stages preceding AMM or IMF (Figure 2). As prefibrotic CIMF-0 is a contradiction of terms and MF is not idiopathic but secondary seen in various MPDs, Georgii *et al*[24] replaced the term CIMF by CMGM as the third entity of prefibrotic MPD different from ET and PV at the bone marrow pathology level (Hannover Bone Marrow Classification of MPD). The prefibrotic stage of CMGM or CIMF precedes the fibrotic stages of AMM (Figure 2) and initially present as primary megakaryocytic and granulocytic myeloproliferation (PMGM) defined by Michiels and Thiele in Table 4. PMGM is characterized by a specific disturbance of a hypercellular bone marrow with striking abnormalities of megakaryocyte maturation (dysmegakaryopoiesis) (Figures 6-10), which consist of variations in size including giant forms and deviations of the nuclear-cytoplasmic ratio accompanied by bulbous and hyperchromatic cloud-like nuclei, which are not seen in ET and PV. Thiele *et al*[29] in their 1999 Cologne Clinical and Bone Marrow Classification of the MPDs used the term prefibrotic chronic IMF (CIMF-0) for this third CMGM MPD entity. Prefibrotic CIMF/CMGM is typically featured by hypercellular ET associated with megakaryocytic, granulocytic myeloproliferation (MGM) at the bone marrow level with no or slight increase of reticulin fibrosis (RF) in the Gomorri's silver or Gordon Sweet stain of bone marrow biopsy specimen (Table 4, Figure 2). With the advent of the JAK2^{V617F} mutation, Michiels distinguish in this report two variants of MGM (Figure 2): JAK2^{V617F} mutated ET due to MGM (ET.MGM) (Table 2, Figure 4) and JAK2 wild type ET associated with primary MGM (PMGM, Table 4, Figures 6-8 and 10). ET associated with JAK2 wild type PMGM (Table 4, Figure 2) is not preceded by any variant of JAK2 or MPL mutated ET or PV.

Table 2 2008 World Health Organization and European Clinical, Molecular and Pathological criteria for the diagnosis and classification of JAK2^{V617F} mutated essential thrombocythemia into 3 stags or phenotypes: important to differentiate because natural history differs

Clinical and molecular criteria	WHO bone marrow criteria
ET stage 1	Normocellular ET
Platelet count of > 350 × 10^9/L and the presence of large platelets in a blood smear in all stages of ET	Predominant proliferation of enlarged megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis
Presence of JAK2^{V617F} mutation	No progression to post-ET myelofibrosis
ET stage 2	Prodromal PV
Platelet count of ⩾ 350 × 10^9/L and normal hematocrit: male < 51%, female < 48% erythrocytes < 6 × 10^{12}/L	Increased cellularity with trilineage myeloproliferation (*i.e.*, panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes No pronounced inflammatory reaction (plasmacytosis, cellular debris). Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis
Presence of JAK2^{V617F} mutation	Progression to overt PV during follow-up
Low serum EPO level and/or increased score for leukocyte alkaline phosphatase	
Spontaneous EEC	
ET stage 3	ET.MGM
Platelet count of ⩾ 3500 × 10^9/L and no signs of leuko-erythroblastosis	Increased cellularity due to MGM and normal or relative reduction of erythroid precursors with various degrees pleiomorphic loosely clustered megakaryocytes containing dysmorphic (not cloud-like) nuclei and maturation defects
Erythrocytes < 6 × 10^{12}/L	No or slight RF (RF 0 or 1)
Presence of JAK2^{V617F} mutation	Progression to post ET myelofibrosis
Slight splenomegaly on ultrasound and no anemia Hb > 12 g/dL	
No preceding or allied of CML, PV, RARS-T or MDS	

Masked myeloproliferative neoplasms: normal platelets, leukocytes and hematocrit, but slight splenomegaly on echogram with the presence of JAK2^{V617F} mutation and/or a World Health Organization (WHO) bone marrow is rare (rather frequent in patients with splanchnic vein thrombosis and/or Budd Chiari syndrome). ET: Essential thrombocythemia; PV: Polycythemia vera; ET.MGM: ET due to megakaryocytic, granulocytic myeloproliferation; EEC: Endogenous erythroid colony; CML: Chronic myeloid leukemia; RARS-T: Thrombocythemia associated with refractory anemia with increased ringed sideroblasts; RF: Reticulin fibrosis.

Table 3 The 2008 World Health Organization and European Clinical, Molecular and Pathological criteria for the diagnosis of polycythemia vera and diagnostic differentiation between polycythemia vera and congenital or acquired erythrocytosis

Clinical and molecular criteria	Pathological criteria (WHO)
Major PV criteria	P1. Early PV
A0. Early PV. Hematocrit in the upper limit of normal: Ht: 0.45 to 0.51 in male and 0.43 to 0.48 in female, Erythrocytes < 6 × 10^{12}/L	Increased cellularity of bone marrow predominantly due to increased erythropoiesis and loose clusters of large megakaryocytes with hyperlobulated nuclei. No or slight increase of granulopoiesis and RF
A1. Classical WHO defined PV: Hematocrit > 0.51/> 0.48 in male/female, Erythrocytes > 6 × 10^{12}/L	P2. Overt PV
A2. Presence of JAK2^{V617F} mutation (sensitivity 95%) or exon 12 mutation	Hypercellular (75%-100%) bone marrow due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorph) megakaryocytes with hyperlobulated nuclei. Absence of stainable iron
A3. Low serum EPO level and/or spontaneous endogenous erythroid colony formation	
Minor MPD criteria	P3. Erythrocytosis
B1. Persistent increase of platelet count: grade Ⅰ: 400-1500, grade Ⅱ: > 1500	Selective increase of erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering of megakaryocytes in primary or secondary erythrocytosis
B2. Granulocytes > 10 × 10^9/L or Leukocytes > 12 × 10^9/L and/or raised LAP-score or increased PRV-1 expression in the absence of fever or infection	Grading of RF (RF 0, 1, 2, 3)
B3. Splenomegaly on palpation or on ultrasound echogram (> 12 cm length in diameter)	Grading of reticulin and collagen fibrosis; myelofibrosis MF grade 1, 2 and 3

World Health Organization (WHO) and European Clinical, Molecular and Pathological (ECMP) criteria criteria for early and overt polycythemia vera (PV). A0, A2, B1 and P1 establish prodromal PV (ET stage 2) PV ECMP stage 0, or masked PV; A1, A2, P1 and none of B establish so-called idiopathic erythrocytosis or polycythemic PV ECMP stage 1. A1, A2, P2 and one or more of B establish WHO defined classic and advanced PV ECMP stage 2 and 3. A1 and P3 with normal or increased values of serum EPO is consistent with congenital or secondary erythrocytosis. A3 confirms early and overt PV without the need of red cell mass measurement for clinicians who do not have access to a hematopathologist expert in myeloproliferative neoplasms. MPD: Myeloproliferative disorders; LAP: Leukocyte alkaline phosphatase; MF: Myelofibrosis; RF: Reticulin fibrosis.

The diagnosis of 2008 WHO fibrotic primary MF (PMF) (Figure 2) is identical to fibrotic stages of PMGM as based on the presence of at least 2 minor criteria and typical bone marrow features including: (1) dense clusters

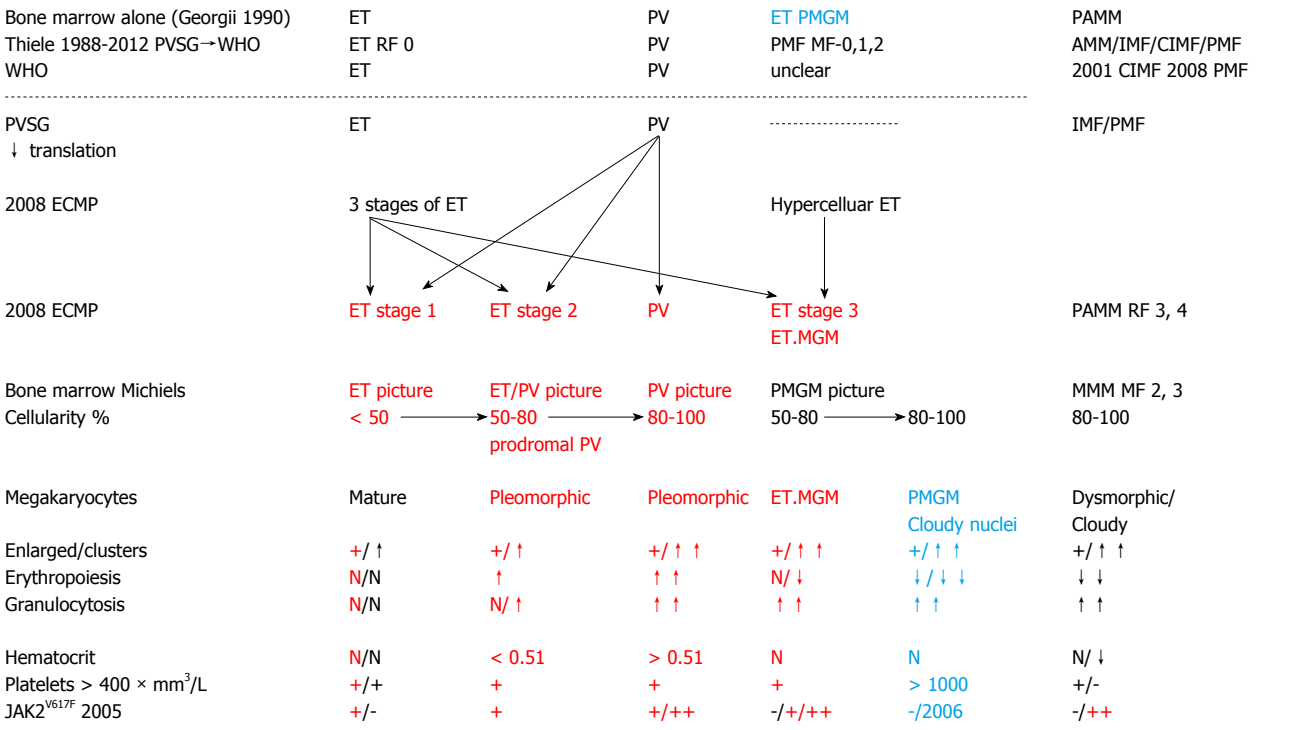

Figure 2 Bone Marrow diagnosis alone: chronic megakaryocytic granulocytic myeloproliferation by Georgii *et al*[24] *vs* chronic idiopathic myelofibrosis by Thiele *et al*[29,75], and comparative World Health Organization and European Clinical, Molecular and Pathological criteria for prefibrotic essential thrombocythemia, polycythemia vera and primary megakaryocytic and granulocytic myeloproliferation or chronic idiopathic myelofibrosis/primary myelofibrosis or agnogenic myeloid metaplasia. Translation of Polycythemia Vera Study Group (PVSG) and 2008 World Health Organization (WHO) defined essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF), chronic megakaryocytic granulocytic myeloproliferation (CMGM) or primary megakaryocytic and granulocytic myeloproliferation (PMGM) according to European Clinical, Molecular and Pathological (ECMP) criteria subdivided in $JAK2^{V617F}$ mutated ET, prodromal PV, overt PV and ET.MGM (red) *vs* prefibrotic PMGM (blue) and 2 types of normocellular ET ($JAK2^{V617F}$ + left red, MPL^{515} + black). PMF: Primary myelofibrosis; AMM: Agnogenic myeloid metaplasia; ET.MGM: ET due to megakaryocytic, granulocytic myeloproliferation; MF: Myelofibrosis.

of large dysmorphic megakaryocytes with immature cloud-like nuclei not seen in ET and PV; (2) increased and normal maturation of granulopoiesis; and (3) various degrees of MF, consistent with fibrotic stage of AMM (Figure 2, clinical stage C2 and C3 in Table 4). Fibrotic stage of PMF is also observed in post-ET MF and post-PV MF (Figure 2).

WHO-ECMP CRITERIA TO DIAGNOSE AND CLASSIFY MPD

The 2008 WHO[21,22] classification of the MPNs ET, PV and PMF is a very important step forward as compared to the PVSG diagnostic MPD criteria for ET, PV and AMM[7-10], but do not meet the needs in daily practice for four reasons[18-20]. First, 2008 WHO criteria for ET only define normocellular ET (WHO-ET), and the diagnosis of ET type 2 with features of early PV (prodromal PV) in blood and bone marrow but normal RCM and erythrocytes ($< 6 \times 10^{12}$/L) is suggested by the 2008 WHO but not clearly defined. Second, the 2008 WHO defined ET include ECMP defined $JAK2^{V617F}$ positive normocellular ET (WHO-ET), prodromal PV and ET.MGM, MPL^{515} positive ET (Figure 2 middle part in red), as well as JAK2 wild type PMGM (Figure 2 right in blue). There is good evidence that $JAK2^{V617F}$ positive hypercellular ET with no leukoerythroblastosis but with increased megakaryocytic granulocytic myeloproliferation (ET.MGM) is featured by reduced erythropoiesis and loose clusters of slight to moderate dysmegakaryopoiesis is rather frequent (ET.MGM, Table 2)[40]. Third, the diagnostic differentiation between $JAK2^{V616F}$ positive ET.MGM without leukoerythroblastosis in Table 2 and JAK2 wild type PMGM without leukoerythroblastosis is clinically relevant and not addressed by the 2008 WHO classification. Fourth, the 2008 WHO classification disregard the importance of increased *vs* normal or decreased erythrocytes, leukocytes, LAP score, platelets and spleen size for diagnosis, classification and staging of thrombocythemia in MPNs of various molecular etiology. Simple tests like blood cell counts including platelets, leukocytes, hematocrit and erythrocytes, spleen size on echogram, EEC, and LAP score are even not taken into account to distinguish the latent (masked), early and overt thrombocythemic and erythrocythemic stages of PV from the overt trilinear polycythemic stage of classic PV. These shortcomings of the 2008 WHO MPN criteria prompted us to propose integrated WHO-ECMP criteria for the diagnosis of ET (Table 2), PV (Table 3) and PMF or PMGM (Table 4).

THREE STAGES OF ET

Sustained increase of platelet counts ($> 350 \times 10^9$/L) associated with slight splenomegaly on echogram (> 12 cm),

Table 4 World Health Organization and European Clinical, Molecular and Pathological criteria for diagnosis and staging of primary megakaryocytic granulocytic myeloproliferation, or primary myelofibrosis

Michiels JJ	Clinical criteria (2005)	Thiele J	pathological criteria (2005/2008)	
A1	Hypercellular JAK2/MPL wild type ET and no preceding or allied other subtype of myeloproliferative neoplasm: JAK2^{V617F} or MPL^{515} normocellular ET, prodromal or classical PV, Ph1+ CML or MDS	B1	PMGM and relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky/clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects	
C	Clinical stages	MF	Staging of myelofibrosis	
C1	Early clinical stages			
	Normal hemoglobin or slight anemia, grade Ⅰ: hemoglobin > 12 g/dL	MF 0	Prefibrotic stage PMGM/PMF	RF 0/1
	No, slight or moderate splenomegaly on ultrasound scan or CT	MF 1	Early fibrotic PMGM/PMF	RF 2
	Hypercellular ET, platelets in excess of 400, 600 or even > 1000 × 10^9/L			
	No leuko-erythroblastic blood picture and/or tear drop erythrocytes			
C2	Intermediate clinical stage			
	Anemia grade II: hemoglobin > 10 g/dL	MF 2	Manifest fibrotic PMGM/PMF	RF 3 = RCF
	Definitive leuko-erythroblastic blood picture and/or tear drop erythrocytes	MF 3	Advanced fibrotic PMGM/PMF	RF 4 = RCF
	Splenomegaly, increased LDH			
C3	Advanced clinical stage			
	Anemia grade Ⅲ: hemoglobin < 10 g/L	MF 3	Osteosclerosis	
	Splenomegaly and increased, normal or decreased platelet count			
	Thrombocytopenia, leukocytosis,leukopenia, increased circulating CD34+ cells			

ET: Essential thrombocythemia; PMGM: Primary megakaryocytic and granulocytic myeloproliferation; CT: Computed tomography; LDH: Lactodehydrogenase; PV: Polycythemia vera; CML: Chronic myeloid leukemia; PMF: Primary myelofibrosis; MF: Myelofibrosis; RF: Reticulin fibrosis; RCF: Reticulin/collagen fibrosis.

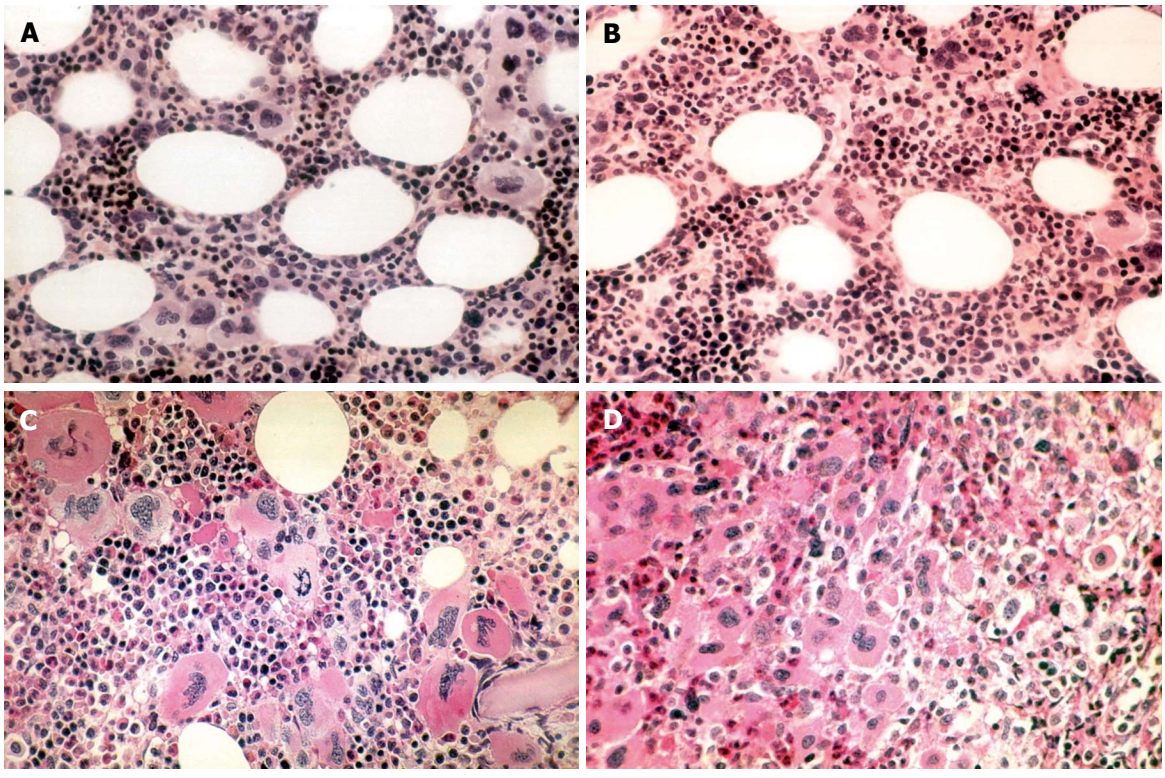

Figure 3 Bone marrow histology features in essential thrombocythemia and polycythemia vera patients. A: Normocellular essential thrombocythemia (ET) bone marrow histology [World Health Organization (WHO)-ET] with increase of clustered pleomorphic megakaryocytes similar as in prodromal and overt polycythemia vera (PV); B: ET/PV bone marrow histology with pleomorphic megakaryocytes and increased cellularity due to increased erythropoiesis as can be seen in WHO and European Clinical, Molecular and Pathological defined prodromal PV and overt PV patients; C: PV bone marrow histology with increased cellularity due to increased erythropoiesis/granulopoiesis and increase of clustered pleomorphic megakaryocytes and no increase of reticulin fibrosis; D: Advanced PV bone marrow histology with dense clustered pleomorphic/dysmorphic megakaryocytes (not cloud-like) and increase in reticulin fibrosis grade 2.

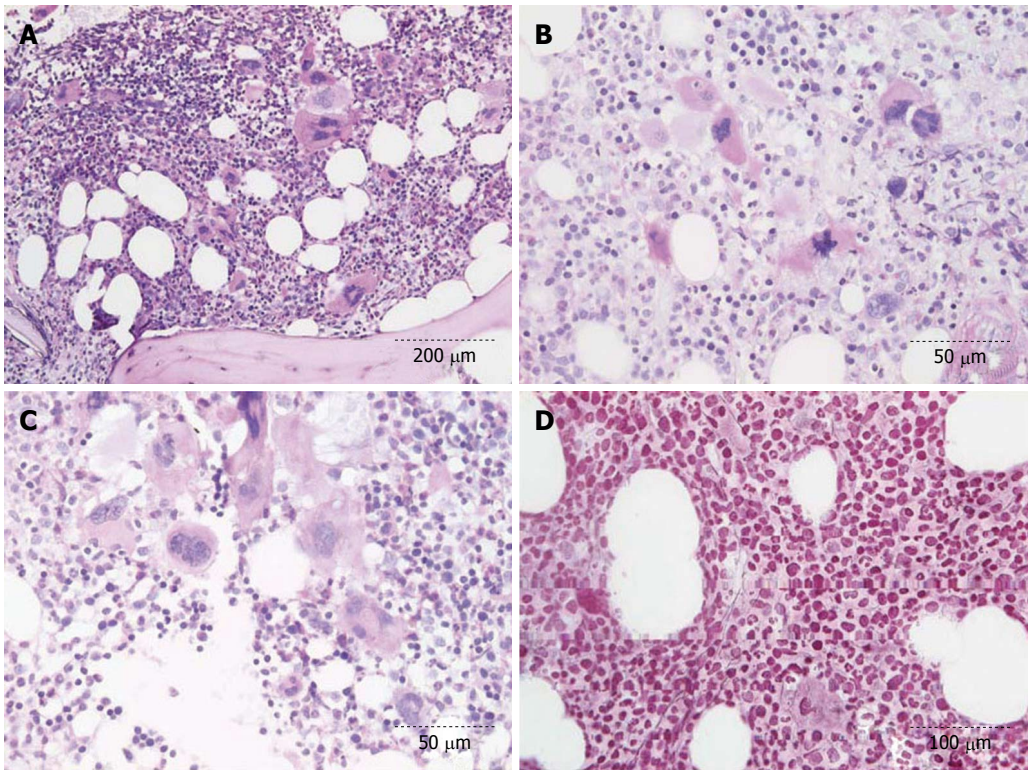

Figure 4 JAK2^{V617F} mutated essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation with slight splenomegaly (spleen 16 cm on echogram) and a hypercellular megakaryocytic granulocytic bone marrow and clusteried pleomorphic clumpsy megakaryocytes with dysmorphic (not cloud-like) nuclei: prefibrotic essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation. A-C: JAK2^{V617}-positive essential thrombocythemia due to megakaryocytic, granulocytic myeloproliferation (ET.MGM) featured by hypercellular ET due to increased megakaryocytic granulocytic myeloproliferation and the presence of pleomorphic/dysmorphic megakaryocytes (not cloud-like); D: Reticulin fibrosis grade 1, myelofibrosis grade 0.

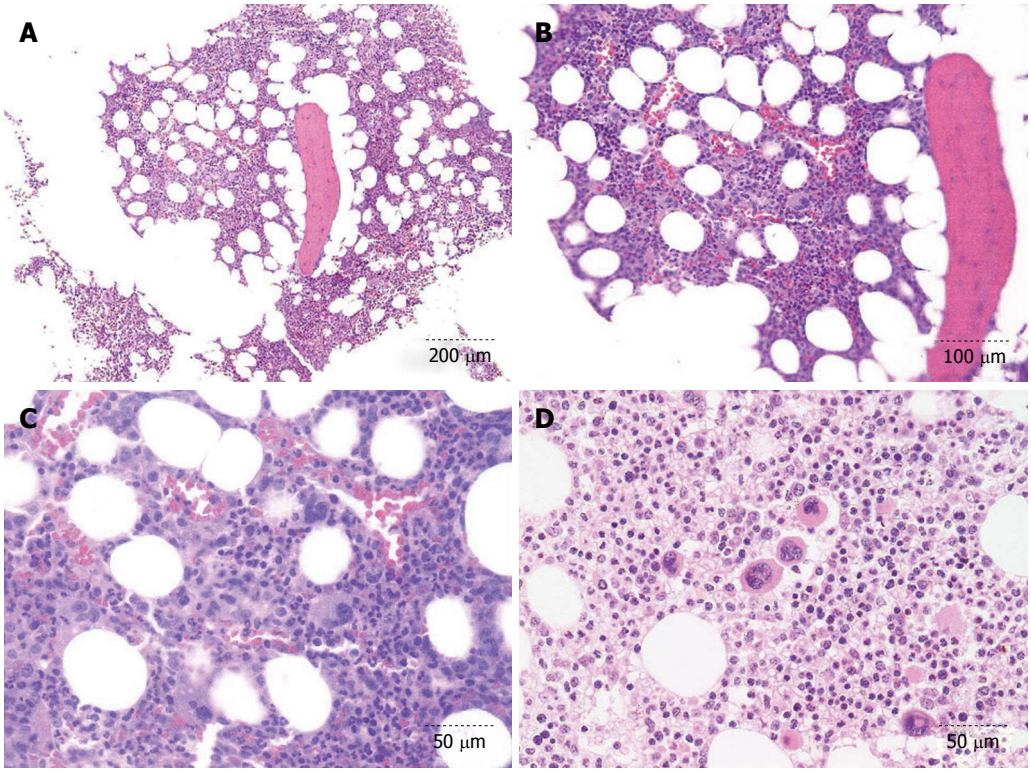

Figure 5 Forty-three-year-old female with positive polycythemia vera (platelets 405 × 10^{9}/L, low serum erythropoietin, leukocyte alkaline phosphatase score 283, hematocrit 0.52, erythrocytes 6.1 × 10^{12}/L, increased red cell mass) with a diagnostic essential thrombocythemia/polycythemia vera bone marrow picture. Such essential thrombocythemia (ET)/polycythemia vera (PV) pictures are regularly seen in prodromal PV and overt PV. A-D: JAK2^{V617F} positive early stage PV with a ET/PV bone marrow histology with loose clusters of pleomorphic megakaryocytes.

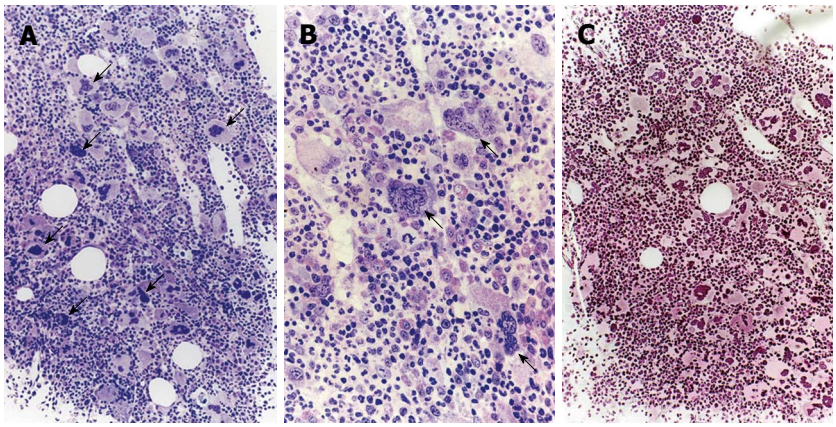

Figure 6 JAK2 wild type hypercellular essential thrombocythemia with platelet counts of 2180 × 10^9/L, no splenomegaly, normal lactodehydrogenase and normal white blood cell differential counts with a characteristics picture of prefibrotic primary dysmegakaryocytic granulocytic myeloproliferation. A, B: JAK2 wild type hypercellular essential thrombocythemia with a typical primary megakaryocytic and granulocytic myeloproliferation bone marrow histology with the presence of abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky, clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects; C: Reticulin fibrosis grade 0. Arrows indicate: Immature dysmorphic megakaryocytes with cloud-like nuclei.

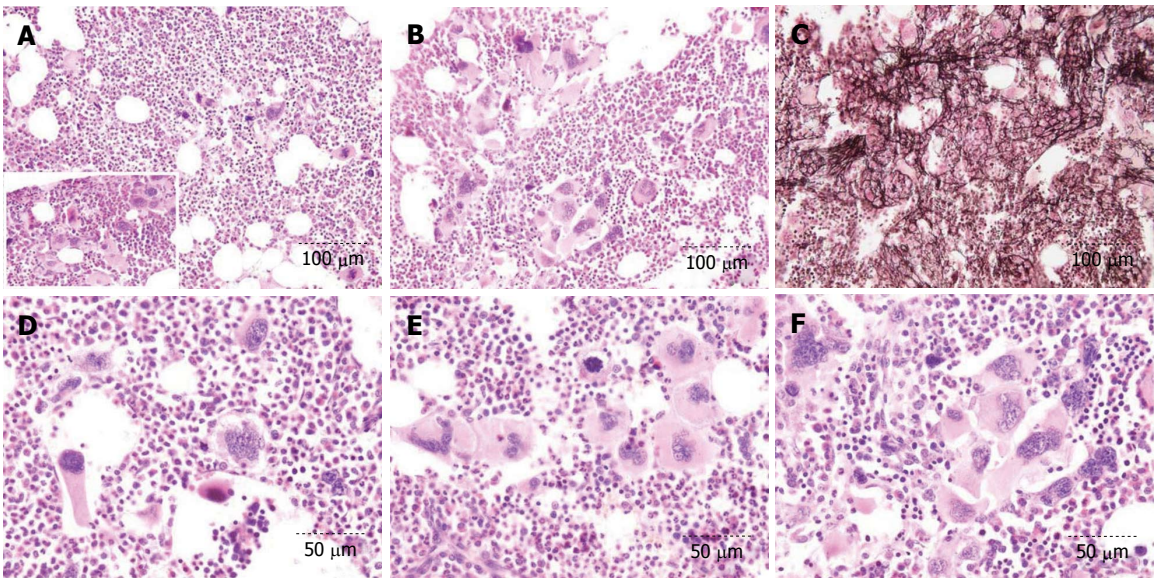

Figure 7 Thirty-seven-years old woman (asymptomatic except fatigue) with JAK2 wild type hypercellular essential thrombocythemia: platelets 1205 × 10^9/L, Hb 12.5 g/dL, erythrocytes 4.9 × 10^{12}/L, leukocytes 18 × 10^9/L, slightly increased lactodehydrogenase, no splenomegaly on palpation as the presenting features of primary megakaryocytic and granulocytic myeloproliferation (Table 5). A, B, D-F: JAK2 wild type hypercellular bone marrow histology due to primary megakaryocytic and granulocytic myeloproliferation with the presence of clustered atypical giant to medium sized dysmorphic megakaryocytes containing bulky (cloud-like) hypolobulated nuclei and definitive maturation defects; C: Reticulin fibrosis grade 2.

normal erythrocytes (< 6 × 10^{12}/L) obviating the need of RCM, normal or increased leukocytes (> 12 × 10^9/L) with normal erythrocyte sedimentation rate (ESR) is suspicious of myeloproliferative ET in the absence of any cause for reactive thrombocytosis. Pre-treatment bone marrow biopsy is needed as the final step in the diagnostic workup to correctly classify the $JAK2^{V617F}$ positive and JAK2 wild type thrombocythemias in various prefibrotic MPDs. The presence of giant platelets in a peripheral blood smear and clustered large or giant mature megakaryocytes in bone marrow smear and biopsy are the pathognomonic clues to the clinical diagnosis of ET. PVSG defined ET without leukoerythroblastosis includes three phenotypes of ET when the WHO-ECMP criteria are applied (Table 2)[18-22]. The WHO-ECMP criteria classify clinical ET as normocellular ET (WHO-ET), prodromal PV mimicking ET, and ET associated with a prefibrotic MGM bone marrow picture without features of leukoerythrocytosis and extramedullary hematopoiesis (ET. MGM) and ET associated with PMGM. The screening for $JAK2^{V617F}$ mutation is very helpful in the diagnostic workup of MPN patients[41-63]. Prodromal PV patients carry the $JAK2^{V617F}$ mutation (Figures 3 and 4). Only half of the patients with PVSG or WHO defined ET carry the $JAK2^{V617F}$ mutation (sensitivity 50% to 60%) and only a very few carry the MPL^{515} mutation[59,60]. A typical MPN bone marrow histology (either ET, PV or PMGM) excludes reactive thrombocytosis, congenital or secondary eryth-

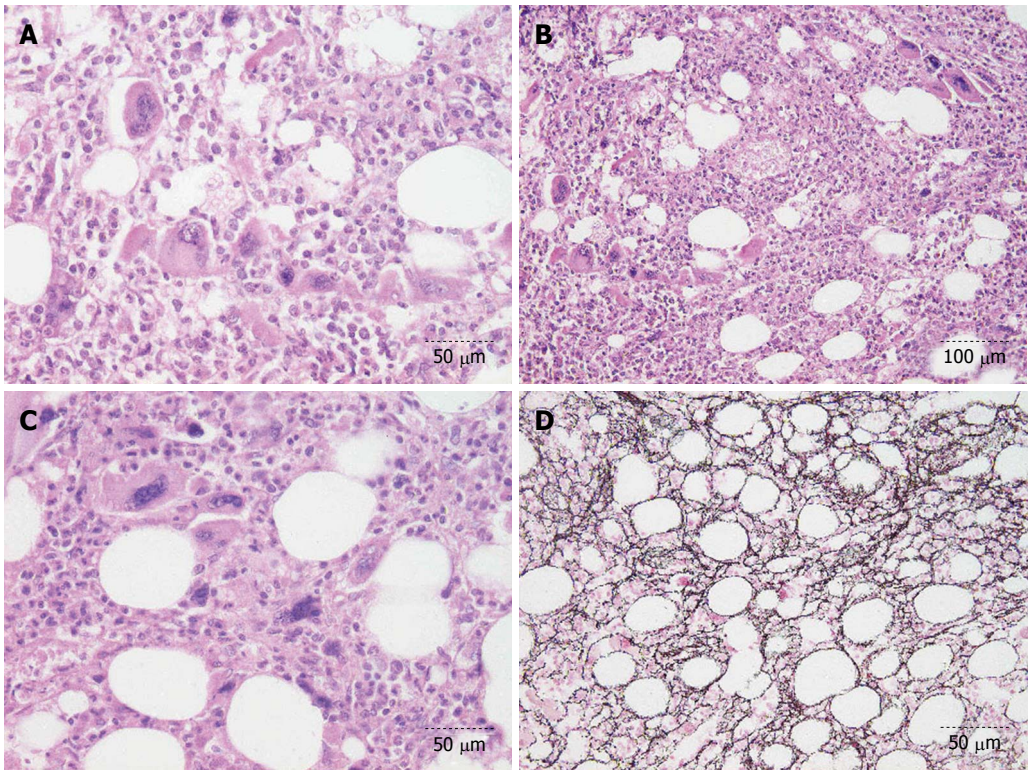

Figure 8 Chronic megakaryocytic granulocytic myelosis according to the Hannover Bone Marrow Classification at time of diagnosis in 1995, and JAK2 wild type primary megakaryocytic granulocytic myeloproliferation according to World Health Organization and European Clinical, Molecular and Pathological criteria in 2006. A-C: Hypercellular bone marrow histology with the presence of Abnormal clustering and increase in atypical giant to medium sized dysmorphic megakaryocytes containing bulky/clumsy (cloud-like) hypolobulated nuclei and definitive maturation defects; D: Reticulin fibrosis grade 2, myelofibrosis grade 1.

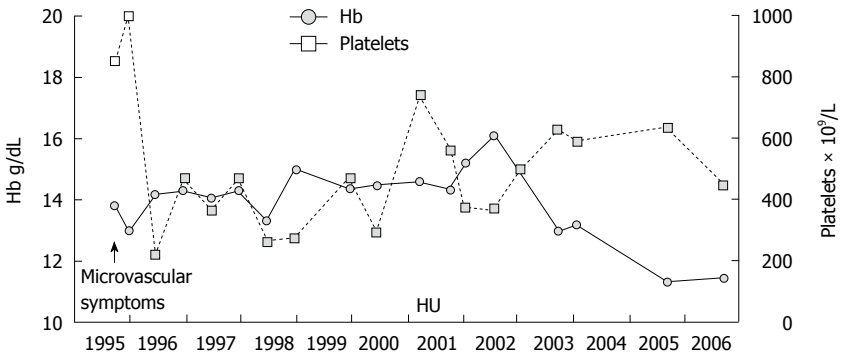

Figure 9 The case of primary megakaryocytic and granulocytic myeloproliferation in Figure 7, who presented in 1995 with microvascular circulation disturbances treated with hydroxyurea for 11 years complicated by mild anemia at platelet counts of 600 × mm^3/L after 10 years of hydroxyurea (HU) for 10 years (1996-2006).

rocytoses, CML, and thrombocythemia associated with refractory anemia with increased ringed sideroblasts[61-63]. WHO-ECMP defined prefibrotic JAK2 wild type PMGM is featured by a hypercellular bone marrow due to pronounced granulopoiesis and dominated by dense clusters of dysmorphic megakaryopoiesis with atypical immature megakaryocytes which are conspicuously enlarged due to increase of nuclear and cellular size with bulky and irregular, round-shaped (cloud-like) nuclei (Table 4, Figures 6-8).

PV *VS* PRIMARY OR SECUNDARY

Characteristic features suspicious for PV include increased hematocrit (> 0.51), increased erythrocytes (> 6×10^{12}/L), slight splenomegaly, increased leukocytes (> 12×10^9/L) or LAP score with normal ESR, an increased platelets (> 400×10^9/L) (Table 3). The detection of JAK2^{V617F} in granulocytes with sensitive polymerase chain reaction (PCR) techniques plays a key-role in the diagnostic work-up of patients with suspected PV (Table 3)[64,65]. In the context of erythrocytosis the presence of the JAK2^{V617F} mutation has a sensitivity of 95% and a positive predictive value of 100% for the diagnosis of PV, and excludes CP and SE without the need of RCM measurement (Figure 2)[18]. In the context of a JAK2^{V617F} positive erythrocythemia (hematocrit > 0.51 in males and > 0.48 in females) the presence of

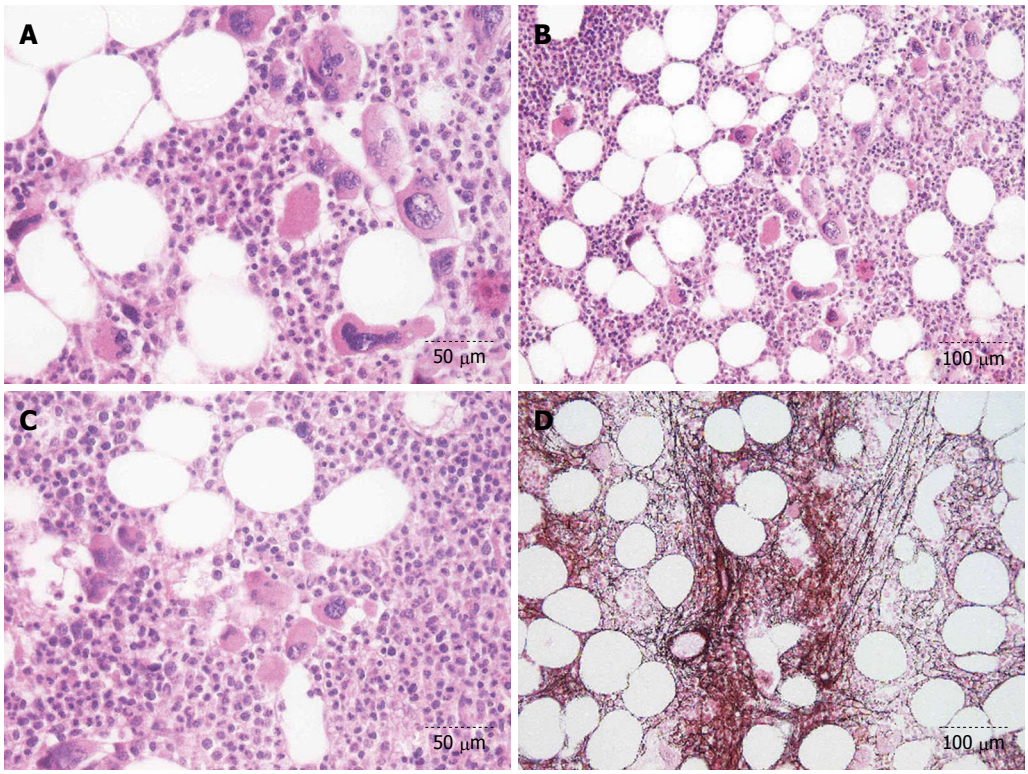

Figure 10 Essential thrombocythemia case diagnosed in 1995 as chronic megakaryocytic granulocytic myelosis, and as JAK2 wild type primary megakaryocytic granulocytic myeloproliferation in 2006 (Figure 8) was complicated by slight anemia and increased bundles of reticulin fibrosis grade 2 after 10 years of hydroxurea treatment (Figure 9). A-C: Bone marrow histology findings in 2006 show thightly clustered immature megakaryocytes with low degree of dysmegakaryopoiesis and cloud-like nuclei. Sometimes the nuclei have an irregular contour and no real hyperchromasia; D: Increase in reticulin fibrosis with many cross-sections grade 2/myelofibrosis grade 1 (Table 5).

large platelets in peripheral blood smear, large megakaryocytes in smears from aspirated bone marrow, low serum EPO, ferritin and slight splenomegaly on echogram are diagnostic for prodromal or overt PV showing a typical ET/PV or PV bone marrow histology picture (Figure 2). As compared to bone marrow histopathology, EEC and serum EPO levels are specific but not sensitive enough to differentiate between myeloproliferative PV, primary erythrocytosis and SE[66]. EEC and serum EPO levels do not differentiate between prodromal PV (normal RCM and normal erythrocyte count) *vs* classic PV (increased erythrocyte count > 6×10^{12}/L and RCM). EEC in the clinical research setting surely will contribute to a better understanding of the role of $JAK2^{V617F}$ in the etiology of heterozygous *vs* homozygous mutated MPN. Pre-treatment bone marrow histology is very insightful and the most powerful tool to stage PV, and to differentiate trilinear hypercellularity in PV from an isolated increase of erythropoiesis in CP and SE with a specificity and sensitivity approaching 100% (Table 3)[67-70]. In SE[69,71] and in CP due to a gain of function mutation in the Epo-receptor, the megakaryocytes are of normal size and morphology and there is no tendency to cluster[72,73]. Differential diagnosis of JAK2 wild type PV, early erythrocythemic PV and idiopathic erythrocytosis (increased RCM and erythrocytes) is problematic and can best be solved by the combined use of bone marrow histology and molecular screening including $JAK2^{V617F}$, JAK2 exon and MPL^{515} mutations obviating the need to measure RCM. About 5% of WHO defined PV patients are $JAK2^{V617F}$ negative and half of them may carry a JAK2 exon 12 mutation[57,58]. Scott *et al*[57] identified JAK2 exon 12 mutations in 10 erythrocytosis patients with increased RCM but negative for the $JAK2^{V617F}$ mutation, which according to PVSG criteria could be diagnosed as PV in 6 and idiopathic erythrocytosis in 4. Pre-treatment bone marrow biopsies in 5 patients carrying one of the JAK2 exon 12 mutations showed characteristic erythroid hyperplasia with slight morphological abnormalities of the megakaryocyte in the study of Scott *et al*[57]. A recent report, 5 cases of $JAK2^{V617F}$ negative PV carrying exon 12 mutation (F537-K539delinsl or N542-E534del) were diagnosed as idiopathic erythrocytosis with increased hemoglobin and hematocrit, low serum EPO, normal platelet and leukocyte counts, no or palpable spleen and a typical hypercellular bone histopathology predominantly due to erythroid hyperplasia and clusters enlarged megakaryocytes with hyperploid nuclei was observed in 2 cases[58].

ROLE OF JAK2 AND MPL MUTATIONS IN THE ETIOLOGY AND PROGNOSIS OF MPN

Applying allele-specific PCR analysis in PVSG-defined MPD patients, a high frequency of the $JAK2^{V617F}$ muta-

tion of 95% (92%-97%) is described in PV, and a lower frequency of 53% (49%-57%) in ET and 52% (44%-55%) in MF (post PV, post ET and PMF)[18,74]. Only 3%-4% of ET, 24%-27% of PV and 6%-18% of MF patients are homozygous for the JAK2^{V617F} mutation[18,72]. Within the JAK2^{V617F}-positive MPNs, good evidences accumulate that the majority of PVSG defined ET patients are heterozygous for the JAK2^{V617F} mutation[41,42] and behaves as an indolent slow onset myeloproliferation of mature enlarged megakaryocytes with no progression to homozygosity and MF during long-term follow-up[43-46]. As compared to JAK2^{V617F}-negative ET, the presence of JAK2^{V617F} mutation has been significantly associated with higher hemoglobin level, higher leukocyte counts and less pronounced thrombocytosis[43-48]. Two studies showed that erythroid burst forming unit colonies are already homozygous for the JAK2^{V617F} mutation in PV patients with a heterozygous pattern of JAK2^{V617F} in their peripheral blood granulocytes[41,42]. Homozygous JAK2^{V617F} PV and PMF refers to a more rapid onset and slowly progressive disease in about one third during long-term follow-up[49-52]. The percentage of JAK2^{V617F} mutation and progression from heterozygous to homozygous due to mitotic recombination of chromosome 9p (loss of heterogeneity of chromosome 9p: LOH 9p) is strongly correlated with increased LAP score, with the ability to form spontaneous EEC and with progressive post-PV MF[50,53]. Homozygous JAK2^{V617F} PV and PMF patients belong to an advanced stage of MPN and displayed significantly higher hemoglobin at time of diagnosis, increased incidence of aquagenic pruritus, higher LAP scores in granulocytes, and higher rate of fibrotic transformation[49-52]. Homozygous MPN patients are older, had larger spleen, more frequent leukocytosis, and displayed evolution to secondary MF and a significantly higher risk of cardiovascular events as compared to heterozygous and wild type MPN patients. Vannucchi *et al*[53] employed quantitative assays for JAK2^{V617F} allele levels in granulocytes in a prospective study of 175 PV patients at time of diagnosis. The JAK2 mutant allele burden could be quantified as 1%-25%, 25%-50%, 50%-75% and 75%-100% in 57, 50, 34 and 32 PV patients respectively at time of investigation. The burden of JAK2^{V617F} allele was directly correlated with abnormally increased levels of hematocrit, white cell and neutrophil count, LDH and LAP score, spleen size on echogram and with decreased values for serum ferritin, and erythropoietin[53]. The JAK2^{V617F} allele burden nicely correlated with a progressively higher relative risk for aquagenic pruritus, spleen size on echogram, total thrombosis and the need for receiving myelosuppressive therapy[53]. Mechanisms other than the mutated JAK2^{V617F} in exon 14 is observed in a proportion of PV and MF patients displaying a gain of chromosome 9p, mostly due to trisomy 9[54-56]. Campbell *et al*[56] reported that the JAK2^{V617F} mutation was found in all 10 MPN patients with trisomy 9, and in 28 of 29 MPN patients (PV, ET or CMF) with a 20q deletion. The finding of the JAK2 exon 12 mutations in patients with PV or idiopathic erythrocytosis, but not in ET further confirms the strong association between the JAK2 mutations and MPN and clearly demonstrates the pivotal role of JAK2 mutations as pathogenic events in variable phenotypes of MPN[57,58]. MPL^{W515L} and MPL^{W515K} mutations has been found in some ET and MF patients indicating the importance of the MPL signalling pathway in the etiology of clonal MPN[59,60].

DIAGNOSTIC DIFFERENTIATION AND NATURAL HISTORY OF JAK2V617F MUTATED ET, PV AND PMF

We propose to extend the PVSG and WHO criteria into a broader set of integrated WHO-ECMP criteria not only for the diagnosis and classification but also for staging of MPN burden at the peripheral blood, spleen and blood and bone marrow level. Upon application of the integrated set of WHO-ECMP criteria, the JAK2^{V617F} mutated ET comprises three phenotypic manifestations of ET including normocellular ET (WHO-ET) (Figure 3), ET 2 with early features of PV (prodromal PV, Figure 3) and ET.MGM with loose or dense clusters of pleomorphic to dysmorphic megakaryopoiesis in a hypercellular bone marrow due to increased granulopoiesis (Figure 4).

The bone marrow histology of JAK2^{V617F} mutated ET.MGM show different grades of granulocytic hypercellularity, which can appear to overlap with PV (hematocrit < 0.51) presenting with a hypercellular bone marrow with more or less pronounced increase of granulopoiesis. The histology of ET.MGM bone marrow showing slightly dysmorphic megakaryopoiesis and may overlap with that of ET cases with mild hyperplasia of granulopoiesis and/or a mixture of mainly mild dysmorphic megakaryocytes (Table 3). Therefore, we may predict a significant overlap (grey zones of about 20%-30%) between ET.MGM and PV with increased granulopoiesis due to an inter-observer disagreement among hematopathologists.

The prognostic importance of the WHO bone marrow features and grading of MF (Table 5) has demonstrated by Kvasnicka and Thiele in a large retrospective study of 865 PVSG defined normocellular ET patients with a platelet count in excess of 600×10^9 mm^3/L[75,76]. In this study, Kvasnicka and Thiele reclassified PVSG defined ET as normocellular true ET (WHO-ET) in 167, and prefibrotic CIMF in 174 and early fibrotic CIMF-1 in 135 according to WHO bone marrow criteria[75]. WHO-ET patients showed no significant loss of life expectancy compared to significant loss of life expectancy in CIMF 0 and CIMF 1 ($P = 0.0001$). The 15 years relative survival was 84% for WHO-ET compared to 68% for CIMF 0 and 55% for CIMF 1. Interestingly, WHO-ET patients were 10 to 12 year younger compared to CIMF 0 and CIMF 1. A similar, large retrospective study has been performed by Barbui *et al*[77]. A total of 1104 PVSG defined ET patients (platelet count > 600×10^9/L) from seven centers in Italy and the United States diagnosed between 1975 and 2008 were analyzed retrospectively using the 2008 WHO clinical and

Table 5 Grading of reticulin fibrosis and myelofibrosis

Grading[78,79]	Grading of MF[80]	Description of RF and RCF in MF as a secondary event in MPN
Normal RF-0	MF 0	No reticulin fibers, occasional individual fibers or focal areas with tiny amount of reticulin fiber network
RF 1 Slight increase	MF 0	Fine reticulin fiber network throughout much of section and no course reticukin fibers
RF 2 Moderate increase	MF 1	Diffuse fine reticuline network with focal collections of thick course reticulin fibers and no collagenisation
RF 3 = RCF Marked increase	MF 2	Diffuse and dense increase in reticulin with extensive intersections, and presence of collagen fibers and no or minor osteosclerosis
RF 4 = RCF and O OS Dry tap	MF 3 Sclerotic	Diffuse and dense reticulin with with coarse bundles of collagen associated with significant O

MF: Myelofibrosis; RF: Reticulin fibrosis; RCF: Reticulin/collagen fibers; MPN: Myeloproliferative neoplasms; O: Osteosclerosis.

bone marrow criteria. Bone marrow biopsies were evaluated by one pathologist (Dr. Thiele). In this cohort of 1104 PVSG defined ET patients, 891 (81%) were diagnosed as normocellular WHO-ET (JAK2^{V617F} positive 61%); and 180 (16%) as hypercellular ET with prefibrotic PMF (pPMF) bone marrow histology (JAK2^{V617F} positive 58%). The overall survival curves show the expected differences in overall survival between WHO-ET and pPMF similar as shown by Kvasnicka *et al*[76]. When compared to the 2008 Eurostat age- and sex-standardized incidences for all causes of death, there was no or minimal loss of life expectancy in WHO-ET MPN patients. The difference of 15 years overall survival in WHO-ET *vs* pPMF (80% and 59%, respectively) was mainly due to the 15 years leukemia-free survival incidence in WHO-ET and pPMF (0.8% *vs* 12.3%, respectively). There were significant differences in leukocyte counts (8.6×10^9/L *vs* 9.7×10^9/L), LDH (298 mU/mL *vs* 429 mU/mL) and reticulin grade grade 1 (3% *vs* 24%) at time of diagnosis of WHO-ET and pPMF 0/1 respectively. Evolution of pPMF 0/1 into fibrotic PMF 2/3 (PAMM) after 10 and 15 years increased from 0.8% to 9.3% in WHO-ET and from 9.3% to 16.9% in pPMF 0/1. Unfavorable prognostic factors in pPMF include LDH above the upper limit of normal, increase of CD34+ circulating cells, spleen size growth of 0.5 to 1 cm/year, slight anemia hemoglobin < 13 g/dL, and constitutional symptoms. In this study 60% of WHO-ET and pPMF patients carried the JAK2^{V617F} mutation. Evolution of WHO-defined ET into myeloid metaplasia of the spleen with MF and leukoerythroblastosis is very rare and predicted to be rather frequent in JAK2^{V617F}-positive hypercellular ET.MGM or JAK2 wild type PMGM. Large scale collaborative prospective management study of newly diagnosed MPN patients comparing the various degrees of JAK2^{V617F} and MPL^{515} mutation load and JAK2 wild type PMGM are needed.

GRADING OF SECONDARY MF IN MPN

The terms RF and reticulin/collagen fibrosis (RCF) are well established in the literature[24,78-81]. Sequential biopsies indicate that initially there is a diffuse or patchy increase in fine RF fibers admixed with abundant hematopoietic elements in PV and PMGM during long-term follow-up. In sequential bone marrow biopsies, the marrow in fibrotic PV and PMF is replaced by course collagen fibers with a decreasing number of hematopoietic cells. This progression from RF to RCF in the bone marrow biopsy during long-term follow-up may be analogous to the similar wound healing, in which collagen composition changes as time passes (fine reticulin type Ⅲ collagen is replaced by course collagen type I collagen). The precise mechanisms by which cytokines from abnormal neoproliferative hematopoietic clone in the various MPNs do stimulate the host's polyclonal fibroblasts to produce excessive amounts of fine RF fibers and course RCF bundles are out of scope in this review.

Two kinds of fiber qualities can easily be distinguishes by common staining in light microscopy: RF[78,79] and RCF[24,80]. Gomorri's silver staining detects early and course RF and do not stain collagen fibers thereby underestimating advanced RCF MF grade 2 and 3. Collagen fibers stain with Mason's trichrome stains, and are negative in the Gomorri's silver stain. Consequently both Gomorri's stain for RF and trichrome stain for CF are to be used for optimal MF-grading of RF and RCF[78,79] and for grading of MF[80] (Table 5). The evolution of RF into RCF as documented by the combined use of silver and trichrome stains simple means a determinative change from reversible normal reticulin (= RF) into progressed pathological collagen scarring (RCF without or with osteosclerois). Clinically, RCF often results in cytopenia and dry tap, when aspiration is attempted. RF with very early RCF usually do occur without real scarring. Bone marrow aspiration in RF without collagen fibrosis (MF-1) usually does not cause the symptom of dry tap. Advanced MF (RCF = MF 2 and 3[80]) designates pronounced increased collagen fibrosis visible scarring spotted areas and sometimes with foci or larger areas of atrophic hematopiesis in the bone marrow in light microscopy.

MF itself is not a disease because RF and RCF is a secondary event induced by polyclonal fibroblasts in response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and PMF. The presence of RF is well documented in ET, PV, PT, PMGM, CML and in many other conditions. Various degrees of RF is rather rare in normocellular ET (WHO-ET) and does occur in about one third of PV and in

Table 6 World Health Organization and European Clinical, Molecular and Pathological staging of prodromal, classical and advanced polycythemia vera related to therapy

PV, ECMP stage	0		1	2	3	4	5
Michiels ECMP Clinical diagnosis	Erythrocythemic PV	Prodromal PV mimicking ET	Polycythemic PV prefibrotic	Classic PV prefibrotic	Advanced PV PMF stage	Post-PV MF Spent phase PV	Leukemic evolution MDS AL
LAP-score	N/↑	↑	↑	↑	↑/↑↑	Variable	Variable
Red cell mass	↑	N	↑	↑	↑	Variable	N/↓
Serum EPO	N/↓	N/↓	↓	↓	↓	Variable	N/↓
Leukocytes × 10^9/L	< 12	< 12	< 12	N-> 12	> 15	> 20	> 20
Platelets × 10^9/L	< 400	> 400	< 400	> 400	< or > 1000	Variable	Variable
Hemoglobin g/dL (mmol/L)	> 16 (10)	< 16 (10)	> 16 (10)	> 16 (10)	> 16 (10)	N/> 12	< 12
Hematocrit	> 0.51	< 0.51	> 0.51	> 0.51	> 0.51	Variable	N↓
Erythrocytes × 10^{12}/L	> 6	< 6	> 6	> 6	> 6	Variable	N/↓
ECMP bone marrow	Early PV	Pro-PV	Early PV	Trilinear PV	Trilinear PV	Myelofibrosis	AML
Bone marrow cellularity (%)	50-80	50-80	60-90	80-100	80-100	Decreased	Increased
Grading myelofibrosis[57]	RF 0-1	RF 0-1	RF 0-1	RF 0/1, MF 0	RCF 2/3 MF 1/2	RCF 3/4 MF 2/3	No MF
Splenomegaly on palpation	No	No/+	No/+	+	++/+++	/Large	Large
Spleen size, echogram cm	< 12	< 12-15	12-15	12-18	18-> 20	> 20	> 20
Spontaneous EEC+	+	+	+	+	+	+	No
JAK2^{V617F} in granulocytes	+	+	+	+/++	+/++	++	No or +
BFU-e (exon 12)	+(++)	+(++)	+(++)	++	++	++	No
Therapeutic implications	Low risk	Low risk	Low risk	Intermediate risk PV	High risk PV	Post-PV MF Spent phase	Acute leukemia
First line treatment option[82,83]	Aspirin phlebotomy	Aspirin phlebotomy low dose IFN?	Phlebotomy Aspirin Low dose IFN → Complete response	Phlebotomy[1] Aspirin IFN→ if resistant →HU	If IFN resistant→ HU or HU first line	JAK2 inhibitor →Bone marrow transplantation Aspirin?	Chemotherapy Bone marrow transplantation? Supportive
Asp/Phleb[82,83]							
IFN[84-86]							
MPN reductive treatment							
Hydroxyurea[83]							
JAK2 inhibitor[87-90]							

[1] ↑: Increased; ↓: Decreased; N: Normal; +: Present or heterozygous; ++: Homozygous. ET: Essential thrombocythemia; PV: Polycythemia vera; MPN: Myeloproliferative neoplasms; MF: Myelofibrosis; AMM: Agnogenic myeloid metaplasia; IFN: Interferon; EEC: Endogenous erythroid colony; ECMP: European Clinical, Molecular and Pathological; LAP: Leukocyte alkaline phosphatase; RCF: Reticulin/collagen fibrosis; RF: Reticulin fibrosis.

the majority of patients with PMGM during long-term follow-up[24,25]. The easiest way in grading of RF using the reticulin silver stain has been performed by the PVSG and in the recent UK study[78,79]. A scoring system of MF based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) and the bone marrow fiber density (fine or course reticulin and some or course bundles of collagen) has been proposed by Thiele *et al*[80] 2005 (Table 5).

CONCLUSION

The underreported early stages of MPN are currently detected by the combined use of clinical, molecular and pathological markers as recommended by integrated WHO-ECMP MPN criteria for the classification and staging of ET, PV and PMGM (Tables 3-6). A wide diffusion and implementation of WHO-ECMP criteria are awaited to clarify their value in recognizing the prefibrotic stages of MPN and in predicting significant differences in long-term prognosis between JAK2^{V617F} normocellular WHO-ET and ET.MGM *vs* JAK2 wild type hypercellular ET associated with PMGM. The urgent need of prospective evaluation of integrated WHO-ECMP criteria include complete blood cell counts (erythrocytes, leukocytes, platelets, LAP score), spleen size on echogram, JAK2 mutation screening, JAK2 mutation load, serum EPO followed by bone marrow biopsy (Tables 2-4 and 6, Figure 2). This integrated approach by clinicians, scientists, molecular biologists and pathologists thereby creates the great advantage to detect all early thrombocythemic and erythrocythemic stages of ET and PV several to more than 10 years earlier. The proposed WHO-ECMP classification and staging of patients with MPN will be very helpful in predicting the natural history of JAK2^{V617F} mutated ET, PV and ET.MGM patients (Table 6), *vs* MPL^{515} mutated ET, *vs* JAK2 wild type PMGM. The WHO-ECMP criteria surely will have important implications in choosing proper treatment options for the management and prevention of thrombotic and bleeding complications and serious complications of progressive MPN disease burden in prodromal PV and classical PV (Table 6)[81-90]. A primary rigid venesection regimen according to Dameshek[3] aiming at a hematocrit below 0.45 in males and below 0.42 in females according on top of low dose aspirin will reduce the cumulative incidence of minor and major thrombosis from above 50% to less than 2% per patient/year during long-term follow-up[16,81,82]. According to current insights, interferon is the treatment of choice in intermediate stage PV patients[82-86]. High risk PV in terms of high JAK2^{V617F} allele burden, progressive MPN disease, splenomegaly and constitutional symptoms are candidates for myelosuppressive (hydroxyurea) or myeloreductive (JAK2 inhibitor) treatment[87-90].

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CASE REPORT

Management of adult Langerhans cell histiocytosis based on the characteristic clinical features

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Abstract

To find out the most appropriate management, clinical features of 18 cases of adult multisystem langerhans cell histiocytosis (LCH) have been analyzed. The patients comprising of 9 males and 9 females were median age of 36 years, ranging from 18-53 years at diagnosis. Regarding the initial symptoms, 7 patients (2 males and 5 females) showed central diabetes insipidus (CDI) and other endocrine symptoms with thickened pituitary stalk or a mass at the hypothalamic region. Additional 2 patients initiated the disease with CDI with no immediate diagnosis. In the remaining patients, the disease begun with single (n = 3) or multiple (n = 1) spinal bone lesion(s) in 4 patients (all males), with multiple bone lesions in 3 patients (1 male and 2 females), with single skull lesion in one female patient and with ambiguous symptoms including hypothyroidism in the remaining one male patient. We also recognized the correlation between pregnancy/childbirth and LCH in 4 patients. In terms of treatment, 9 patients received systemic immuno-chemotherapy alone, of which the majority received vinblastine-based chemotherapy while 4 received 2-chlorodeoxyadenosine. Five had a combination of immuno-chemotherapy with surgical resection or radiotherapy, 2 had immunotherapy alone, 2 had surgical resection followed by observation alone to date. Three patients received hematopoietic stem cell transplantation after extensive chemotherapy. In terms of outcome, 15 patients are alive (9 with active disease, 6 without active disease), with a median of 66 mo (range 17-166 mo), two died of disease while the remaining 1 lost to follow-up. Based on these results, we think that early diagnosis and rapid introduction of appropriate treatment are essential, in order to overcome the problems relevant to adult LCH.

Key words: Langerhans cell histiocytosis; Adult; Immuno-chemotherapy; 2-chlorodeoxyadenosine; Childbirth

Core tip: Clinical features and treatment in a total of 18 adult patients with langerhans cell histiocytosis (LCH) were reviewed. We found two major groups regarding the initial symptoms; one was central diabetes insipidus and other endocrine symptoms (n = 9) and the other bone diseases (n = 8; 1 skull, 4 spinal and 3 multiple). We also recognized the correlation between pregnancy/childbirth and LCH in 4 patients. Based on the clinical features and outcomes, early diagnosis and rapid introduction of appropriate treatment are essential, in order to overcome the problems relevant to adult LCH.

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INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare lymphoproliferative and granulomatous disease comprising of CD1a-positive LCH cells, T-cell lymphocytes, macrophages, eosinophils and other cells[1-3]. About two-thirds of LCH cases are seen in childhood, with the remaining occurring in adulthood[4]. Information on clinical features in adult LCH patients is limited[5,6]. While pediatric LCH has been extensively investigated, adult LCH remains poorly scrutinized, except for characteristic pulmonary diseases, which is closely related to smoking[7,8]. Epidemiology of non-pulmonary adult LCH is still poorly understood. LCH is an enigmatic disease that is thought to be caused by a pathological combination of oncogenesis and immune dysregulation[1-3]. Aricò *et al*[5] analyzed 274 biopsy-proven adult LCH cases from 13 countries; the mean ages at the onset and diagnosis of disease were 33 years and 35 years, respectively, with single-system LCH (SS-LCH) 31.4% and multisystem disease LCH (MS-LCH) 68.6%. Central diabetes insipidus (CDI) was found in about one third (29.6%) of the patients. The probability of survival in these cases at 5 years post-diagnosis was 92.3%, indicating that the adult LCH is not necessarily a fatal disease, but the highly problematic in adults is the impaired quality of life associated with active disease, particularly of MS-LCH. Based on our previous literature review on 43 Japanese adult cases[4], age distribution showed a peak at 20-40 years of age (31/43 cases, 72%), particularly in females in the 3rd decade. Five cases (11.6%) were older than 55 years. In terms of involved organs, beside lungs (n = 15) which are the most frequently involved organ, bone (n = 11), CDI (n = 10), skin (n = 8), followed by various organs were noted in these adult cases. The LCH in adults excluding isolated pulmonary disease could be comparable with those in childhood; however, the characteristics in adult disease remain elusive. Here, we present a case series on recently treated 18 adult MS-LCH and review clinical features with specific issues relevant to adult patients, including imaging of characteristic findings. Regarding the treatment/outcome, there seems to be a significant progress in recent cases described here, compared to the cases in our previous literature review[4]. In this case series, we also attempted to find out the appropriate therapeutic measures in adult LCH patients.

CASE REPORT

Patients and methods

Adult (> 18 years) LCH cases referred to the authors for treatment and/or consultation during the period of 1999-2012 have been analyzed for clinical characteristics and treatment. Cases of isolated pulmonary LCH were excluded in the analysis. The patients comprising of 9 males and 9 females were median age of 36 years, ranging from 18-53 years at diagnosis. The diagnosis of LCH was confirmed immunohistochemistry [S100 (+), CD1a (+) or CD207 (+)] on the biopsied or resected tumors in all patients. In terms of treatment, besides surgery/radiotherapy or immuno-chemotherapy, all patients with CDI were given nasal desmopressin acetate hydrate (DDAVP) and those with other endocrine symptoms received hormonal replacement therapy. Outcome at the last follow-up was defined as alive with no active disease (ASAD), alive with disease (AWAD), or died. The follow-up period of the 15 patients excluding 1 lost-to follow up and 2 deceased was a median 66 mo (range 17-166 mo). Of these cases, Cases 13-16 were previously published focused on the endocrine problems[9]. Case 17 were included in an analysis of LCH-related neurodegenerative disease[10] and Cases 4 and 17 were also included in a therapeutic trial of LCH[11]. Case 10 was briefly reported as a case of multiple bone lesions[12].

Case 1

A 27-year-old female complained of amenorrhea and polyuria/polydipsia after her second childbirth. She was found to have a mass at the hypothalamic pituitary region (HPR), the biopsy of which revealed LCH. She was treated with radiotherapy (20 Gy) to the CNS mass, oral prednisolone (PSL) and DDAVP. No other LCH lesions developed in this case, thus no other systemic chemotherapy was given. In terms of outcome, she has been lost to follow-up.

Case 2

A 25-year-old female developed amenorrhea and polyuria/polydipsia after her second childbirth. She had a mass at the HPR, and granulomatous lesions at the nasal alar parts as well as thyroid mass, which biopsy revealed LCH. At first she was treated with hydrocortisone, DDAVP and levothyroxine sodium. Four years later, multiple LCH lesions were noted at her scalp, perineal-anal regions, subcutaneous abscess-like lesions at the lumbar area, cervical subcutaneous mass. She was treated with systemic chemotherapy (cyclophosphamide/etoposide/PSL), but remained in a partial remission. Particularly, her recalcitrant fistula-forming subcutaneous lesions were probably related to her poorly controlled diabetes mellitus. Eventually, she died of sepsis-induced disseminated intravascular coagulation (DIC).

Case 3

A 35-year-old female developed multiple masses after childbirth at the right subauricular and subscaplar regions and right 5th rib, which biopsy revealed LCH. She then developed multiple bone lesions at the spine (C2, C4, C5, Th3-4), clivus, occipital bone, as well as subcutaneous

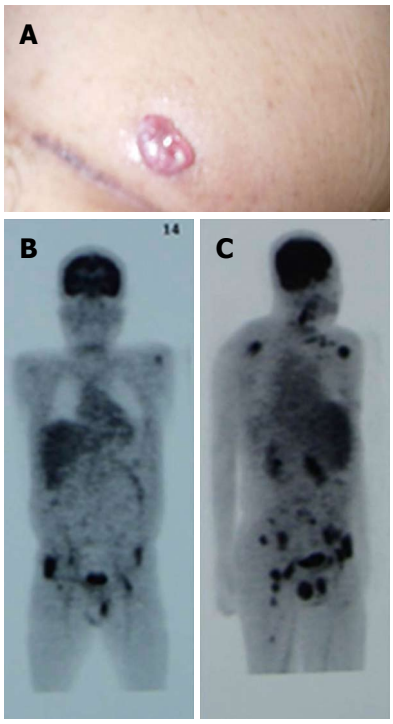

Figure 1 Langerhans cell histiocytosis skin lesions and systemic positron emission tomography imaging. A: Soft papular skin lesion is found at the inguinal area. Biopsy of the lesion revealed the histology of langerhans cell histiocytosis; B, C: Positron emission tomography scan shows multiple lesions, at the scapulas, plevic bones, cervical and inguinal lymph nodes: frontal view (B), lateral view (C).

masses (at her left upper arm and elbow). The lesions at the Th3-4 caused paresis due to spinal compression, which needed laminectomy. She was initially treated with bisphosphonate alone, but thereafter with intensive systemic chemotherapy (JLSG-96 protocol)[13], including vincristine and cytosine arabinoside. However, 2 years later, she needed allogeneic hematopoietic stem cell transplantation (HSCT) because of progressive disease. The patient has currently been alive without active disease (ASAD).

Case 4

A 37-year-old male was found to have CDI, in association with hyper-prolactinemia (seum PRL 56.1 ng/mL). A thickened pituitary stalk was demonstrated on brain magnetic resonance imaging (MRI), which biopsy revealed LCH. Since then he developed multiple bone lesions at spines (cervical, lumbar), bilateral ilium, left sacroiliac joint, and bilateral ribs as well as skin lesions (Figure 1A) and cervical lymphadenopathy. He was treated first with PSL and bisphosphonate followed by systemic chemotherapy (JLSG-96 protocol)[13]. One year later, because of progression of the disease (Figure 1B and C), he received HSCT and has currently been in a state of ASAD.

Case 5

A 40-year-old male complained first of mandibular pain. He was found to have multiple bone lesions (left mandible, left temporal, occipital, bilateral femurs, cervical spines and right petrous bone. Biopsy of the temporal bone lesions revealed LCH. He was treated with irradiation (6 Gy) to the left petrous bone and systemic chemotherapy, including vinblastine (VBL) and PSL followed by the JLSG-96 protocol[13] and bisphosphonate; however, reactivation occurred to C1, C2, right mandible, Th5, bilateral femur distal end. Thereafter, he responded well to the chemotherapy with 2-deoxychloroadenosine (2CDA; cladribine). The patient has currently been in a state of ASAD.

Case 6

A 46-year-old female, who was first noted to have thyroid cysts at age 43, complained of back pain and found to have a compression fracture of Th4. A year later she complained of right rib pain. Positron emission tomography (PET) scan revealed hot spots (SUVmax; 7.0) at the both thyroid lobe (Figure 2A) as well as other numerous hot spots at the deep cervical lymph nodes, right scapula, right ilium as well as right rib. Another year later, right lobe of the thyroid was removed, which was diagnosed as LCH. She also received a resection of right rib, which was also found to be LCH. Since then, she was treated with VBL and PSL; however, treatment was stopped because of VBL neurotoxicity. Thereafter, new bone lesions at the frontal bone, rib, ankle, knee, *etc*. She gave births two children; however, the initiation of LCH was not relevant to her childbirth. The patient has continued to have active disease (alive with active disease, AWAD).

Case 7

A 40-year-old male complained of nuchal and left-shoulder pain without any triggering events. With MRI, he was found to have a mass at the left upper and lower articular processes and part of the left articular arch of C3, which was positive for Ga^{67} bone scintigraphy. This mass was totally resected. Nearly 2 years later, he had a tender and soft swelling at the right sided parietal bone (Figure 2B), which was also positive for Ga^{67} scintigraphy. This tumor was again resected. Both tumors were diagnosed as LCH. Currently at age 46, the patient has been followed up without receiving any systemic chemotherapy and in a state of ASAD.

Case 8

A 40-year-old male was found to have a mass at the left articular arch to the spinous process of C6, which was significantly hot (SUVmax; 6.6) with PET scan (Figure 3). The mass was biopsied, which was diagnosed to be LCH. The CT scan showed the lymphadenopathy at the bilateral axillary as well as inguinal area. Thus, an inguinal node was biopsied, which also showed LCH with complex karyotypes. Past history showed he had a severe atopic dermatitis since age of 20, treated with Protopic (tacrolimus hydrate) ointment. He was treated with systemic chemotherapy [VBL/methotrexate (MTX)/PSL/bisphosphonate]. The patient has currently been treated for active C6 lesion.

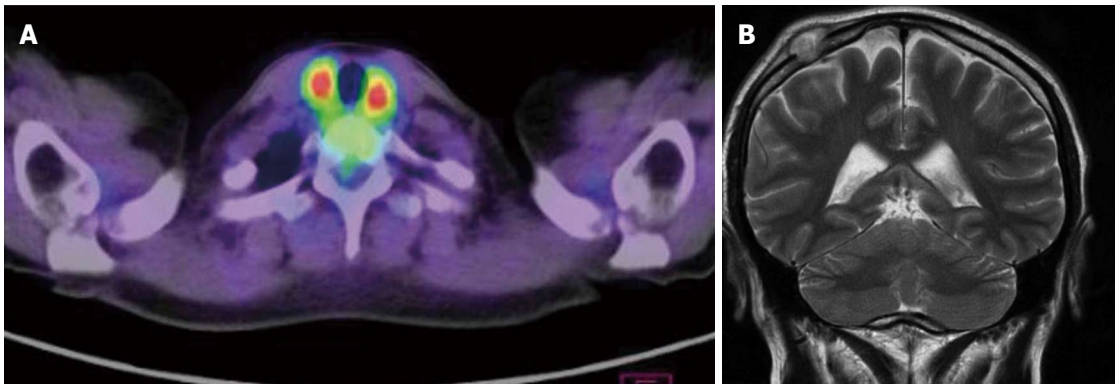

Figure 2 langerhans cell histiocytosis thyroid (A) and skull (B) lesions. A: Positron emission tomography scan shows hot spots (SUVmax = 7.0) at both lobes of thyroid; B: Magnetic resonance imaging (T2W2) shows a mass at the right parietal region.

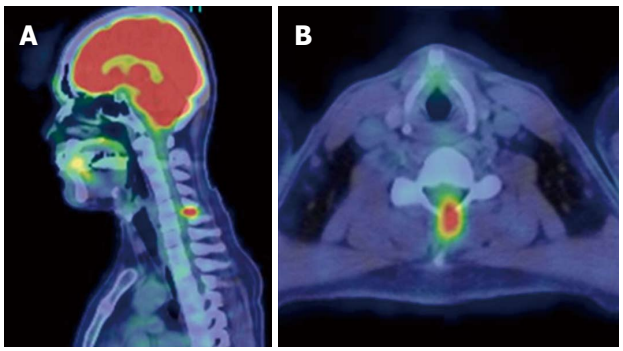

Figure 3 langerhans cell histiocytosis bone lesion at cervical vertebra 6. Positron emission tomography scan shows a hot spot (SUVmax = 6.6) at the spinous process of C6. A: Sagittal view; B: Axial view.

Case 9

A 27-year-old female, who had been treated for atopic dermatitis since childhood, was noted to have a swelling at the right orbit. MRI showed a mass lesion at the right temporal bone extended to the adjacent muscle, which consisted of heterogeneous components (Figure 4A and B). The mass was extensively removed by surgery and the diagnosis of LCH was made. PET scan did not reveal any other suspected lesions. Thus, she was put on observation alone. The patient has been followed up longer than two years without reactivation of the disease. No CDI has occurred and the patient has been in a state of ASAD.

Case 10

A 31-year-old male was first noted to have 1st thoracic spine lesion at age 25, which was biopsied and diagnosed as LCH. Two years later the systemic bone survey revealed a skull lesion, which was surgically removed. Two more years later, he had difficulty in opening mouth and was diagnosed to have a lesion at the mandible bone. Since then he was treated with PSL and bisphosphonate. Nevertheless, he complained of right sided lumbago two more years later due to the involvement of newly appeared iliac bone lesions. Currently the patient has had multiple bone lesions as well as CDI. He needed further systemic chemotherapy for active disease.

Case 11

A 53-year-old female first developed CDI at age of 41. Two years later, she was noted to have a right mandibular bone mass which was totally resected; however, diagnosis remained unknown. Three more years later she developed bone lesions at the frontal to left temporal skull, which caused a diffuse bone defect, including skull and facial bones (Figure 4C), when LCH was diagnosed. With systemic CT scan, she also was noted to have multiple osteolytic lesions at the skull, spine, scapula, rib and pelvis. She received LCH-A1 protocol[6], consisting of VBL and PSL for a year, which induced a new bone synthesis at the frontal bone. More recently, 12 years after the onset of initial symptoms, she has had newly-developed left mandibular osteolysis. She has currently been treated for active disease.

Case 12

A 36-year-old female was noted to have amenorrhea even after stopping milk feeding for her first-born baby. Administration of Gonadotropin-releasing hormone was successful in resuming a menstrual cycle and she gave birth of second child; however, soon after second childbirth she complained of symptoms compatible with CDI, when brain MRI showed a loss of bright spot at the pituitary posterior lobe; in addition, almost a year later, she was found to have a hypothalamic mass with a gadolinium (Gd)-enhanced MRI (Figure 5A). Biopsy of the mass confirmed the diagnosis of LCH. Since the probable compression of cerebrum due to a huge mass caused impaired consciousness, the patient received systemic chemotherapy, first with VBL followed by 2CDA.

Case 13

A 20-year-old girl first complained of polyuria/polydipsia and amenorrhea. Eight months later, she was detected a Gd-enhanced mass at the HPR by a brain MRI. An immediate open biopsy of the mass confirmed the diagnosis of LCH. Initially, irradiation (21 Gy) to the CNS markedly reduced the size of mass. Two years later, the patient was noted to have re-growth of hypothalamic mass, continued amenorrhea, poorly-controlled CDI and generalized cutaneous LCH, which was confirmed by

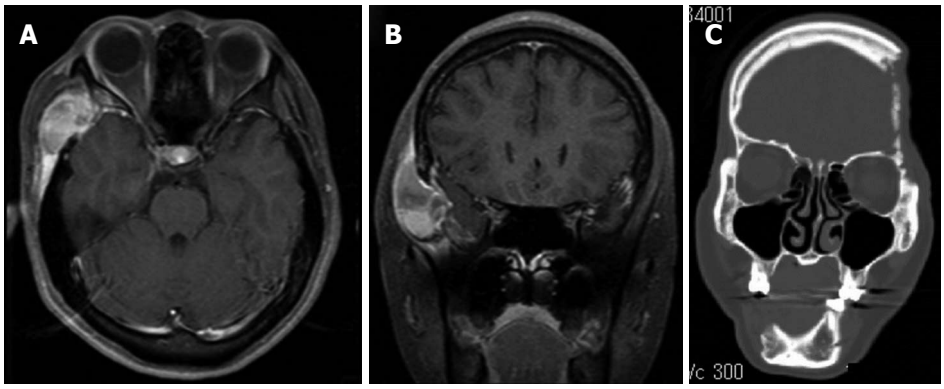

Figure 4 Langerhans cell histiocytosis skull mass (A, B) and lytic skull (C) lesion. A, B: Gadolinium-enhanced magnetic resonance imaging (T1W1) shows a mass with heterodensity at the right temporal area. axial view (A), coronal view (B); C: Computed tomography scan shows extensive lytic bones, at the left temporal bone and mandible. Defect of the right mandible is due to surgical resection.

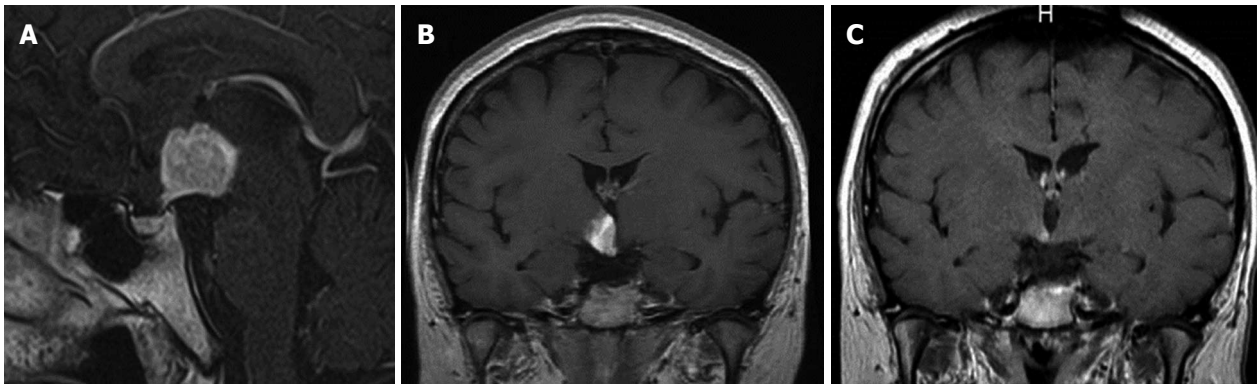

Figure 5 Langerhans cell histiocytosis mass at the hypothalamic area (A) and hypothalamic–pituitary area, comparison of pre and post chemotherapy (B, C). A: Gadolinium-enhanced magnetic resonance imaging (W1T1) shows a large mass at the hypothalamic area. Pituitary stalk is enlarged; B, C: Gadolinium-enhanced magnetic resonance imaging (T1W1) shows (B) a large mass before chemotherapy and (C) a residual mass post chemotherapy. The size of mass was significantly reduced after 2-deoxychloroadenosine treatment.

biopsy. Re-institution of systemic chemotherapy significantly (> 50%) reduced the size of hypothalamic mass. The patient has currently been in a state of ASAD.

Case 14

A 36-year-old female developed CDI in association with amenorrhea. However, the thickened pituitary stalk detected by MRI was put on under observation and not immediately treated. After progression of the thickened pituitary stalk into a significant Gd-enhanced mass at the HPR, a biopsy was performed to reveal typical LCH histology, when the patient had amenorrhea, fatty liver, reduced glucose tolerance. Systemic chemotherapy with 2CDA significantly (> 50%) reduced the mass size (Figure 5B and C). LCH lesions outside the CNS were not found. The patient remains markedly obese and diabetic, with residual active disease.

Case 15

A 38-year-old male was first diagnosed with primary hypothyroidism. Nineteen months later, he developed the symptoms of CDI, fatigue and disturbed consciousness along with disorientation and abnormal behaviors. A brain MRI revealed a Gd-enhanced mass at the HPR, which biopsy did not confirm the diagnosis of LCH. Two years later, osteolytic bone lesions appeared on the right femur and left clavicle, when the LCH was eventually diagnosed by a bone biopsy. The patient received systemic chemotherapy with 2CDA, but the mass size was reduced minimally (< 50%), and he remained significantly obese and diabetic. Four years from the initial CDI symptom, the patient developed retropharyngeal B-cell lymphoma. He has had active diseases of LCH as well as lymphoma.

Case 16

A 46-year-old male first presented with decreased libido and erectile dysfunction seven years after total gastrectomy for gastric adenocarcinoma. Four years later, a Gd-enhanced mass at the HPR was detected. Until then, he had ignored his polydipsia/polyuria symptoms, thus the diagnosis of CDI was delayed. Biopsy of the CNS mass led to a diagnosis of LCH. A systemic MRI survey also revealed multiple spinal involvements. The patient also had loss of concentration and short term memory deficits suggesting mild neurodegenerative disease, which findings were confirmed with brain MRI examination. Eventually, the patients received systemic chemotherapy

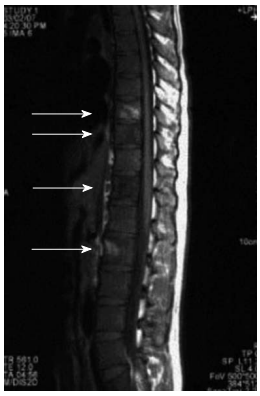

Figure 6 Langerhans cell histiocytosis spinal lesions. Non-enhanced magnetic resonance imaging (T1W1) shows high and low signals at multiple vertebral bodies (arrows).

with 2CDA, which markedly (> 50%) reduced the CNS mass size. However, currently, he has been treated for the regrowth of the CNS mass.

Case 17

A 23-year-old young adult was noted to have cerebellar ataxia and dysarthria. Past history revealed that at age of 16, he had been diagnosed as CDI; however, the exact cause was unidentified. At age 20, he was found to have polycystic lung disease with pneumothorax, followed by a mild ataxia. At age 23, he suffered a traffic accident when he was incidentally found to have a brain disease with a mass at the HPR as well as neurodegenerative disease on MRI performed at the emergency hospital. Eventually, LCH was diagnosed from the biopsy of lung tissues. He received monthly intravenous immunoglobulin therapy for neurodegenerative disease with dexamethasone[10]; however, the patient declined further treatment. Currently, he has remained with progressive neurological symptoms and with active lung disease.

Case 18

An 18-year-old man initially complained of low back pain and cervical mass. MRI revealed multiple spinal bone involvement (Figure 6). The initial diagnosis of LCH was made from the histology obtained by excisional iliac biopsy. A year later, he developed swelling of left cervical lymph nodes. CT scan of the chest also revealed a nodule in the right lung and the enlargement of left upper mediastinal lymph nodes. Histopathology of the biopsied cervical lymph node showed coexistence of two tumorous components; one was LCH and the other tissue of Hodgkin disease with Reed-Sternberg/Hodgkin cells being positive for CD30. The disease responded temporarily to irradiation (36 Gy) and systemic chemotherapy, but became refractory with relapses to the lungs and lymph nodes. Despite autologous followed by allogeneic HSCTs, he died of refractory Hodgkin disease at age of 23.

Summary of the cases

As summarized in Table 1, cases consisted of 3 SS-LCH (all CNS disease) and 15 MS-LCH. Regarding the initial symptoms, 7 (2 males and 5 females) of the 18 patients had CDI and other endocrine symptoms with thickened pituitary stalk or a mass at the HPR. Additional 2 patients initiated the disease with CDI with no immediate diagnosis. In the remaining patients, the disease begun with single (n = 3) or multiple (n = 1) spinal bone lesion(s) in 4 patients (all males), with multiple bone lesions in 3 patients (1 male and 2 females), with localized skull/muscle lesion in one female patient and with ambiguous symptoms including hypothyroidism in one male patient. Thyroid mass was noted in 2 patients. In terms of treatment, 9 patients received systemic immuno-chemotherapy alone, of whom 3 with CNS disease and 1 with multiple bone lesions received 2CDA. Five patients had a combination of immuno-chemotherapy with surgical resection or radiotherapy, 2 had immunotherapy alone, 2 had surgical resection followed by observation alone to date. Three patients received HSCT after extensive chemotherapy. In terms of outcome, 15 patients are alive (9 with active disease; AWAD, 6 without active disease; ASAD) with a median follow-up of 66 mo (range 17-166 mo) and 2 died of disease; 1 from sepsis-induced DIC and the other from progression of Hodgkin disease. The remaining 1 patient is lost to follow-up. As late sequelae, CDI (n = 9), neurodegenerative disease (n = 2) and obesity/diabetes mellitus (n = 3) are noted.

DISCUSSION

Clinical features

To date, adult LCH cases have mostly been reported as case series[14-18]. Here, we add another case series describing the clinical features and discussing the issues specifically relevant to adult LCH. Adult patients may have LCH as a recurrence of childhood LCH as well as *de novo* LCH developing first in adult life. Here described is all the latter type of LCH. None had a history of LCH in childhood.

Two major clinical features of non-pulmonary LCH in adults

It is apparent that there are two major groups; one is a CNS mass with endocrine problems (9/18) and the other is recalcitrant bone lesions (7/18). Both types of disease are histologically non-malignant, but extensive disease causes various impairments leading to the decreased quality of life, and limiting to achieve normal daily life activity.

Endocrine problems

Adult patients are often noted first with endocrine problems such as CDI, amenorrhea, loss of libido and obesity[9]. Particularly, hypothalamic-pituitary disease is the most common CNS manifestation of LCH, which leads to CDI and anterior pituitary hormone deficiencies. CDI is diagnosed from the finding that MRI scan shows absence of the bright spot of the posterior pituitary on

Table 1 Summary of 18 cases of non-pulmonary adult langerhans cell histiocytosis

Cases	Age (yr)/sex	Initial symptoms/signs	Subsequent symptoms/ disease progression	Treatment			Outcome	Follow-up (mo)
				Surgical resection	Radiation	Immune-chemo Rx/HSCT		
1	27/F	CDI/endocrine	None	Y	Y	Y[4]	LTF	-
2	25/F	CDI/endocrine	Nasal/thyroid/skin masses			Y	DOD[1]	216
3	35/F	MBL	MBL			Y/Y	ASAD	132+
4	37/M	CDI	MBL/skin lesions			Y/Y	ASAD	128+
5	40/M	MBL	MBL		Y	Y	ASAD	133+
6	46/F	Thyroid mass/spine (Th4)	MBL/LNs	Y		Y	AWAD	27+
7	40/M	Spine (C3)	Parietal bone	Y			ASAD	72+
8	40/M	Spine (C6)	Inguinal LNs			Y	AWAD	17+
9	27/F	Temporal bone	None	Y			ASAD	26+
10	31/M	Spine (Th1)	MBL			Y[4]	AWAD	108+
11	53/F	CDI	MBL	Y		Y	AWAD	166+
12	36/F	Endocrine	HPR mass			Y[3]	AWAD	28+
13	20/F	CDI/endocrine	Skin		Y	Y	ASAD	156+
14	36/F	CDI/endocrine	None			Y[3]	AWAD	52+
15	38/M	Hypothyroid	CDI/endocrine			Y[3]	AWAD	48+
16	46/M	Endocrine	CDI/MBL/ND-CNS			Y[3]	AWAD	60+
17	23/M	CDI	Lungs/ND-CNS			Y[4]	AWAD	72+
18	18/M	Spine (multiple)	LNs/Lungs		Y	Y/Y	DOD[2]	72

[1]From disseminated intravascular coagulation; [2]From Hodgkin disease; [3]Systemic chemotherapy with 2-deoxychloroadenosine; [4]Steroid alone with or without bisphosphonate. CDI: Central diabetes insipidus; MBL: Multiple bone lesions; HPR: Hypothalamic pituitary region; LNs: Lymph nodes; ND-CNS: Neurodegenerative central nervous system disease; Y: Yes; LTF: Lost to follow-up; ASAD: Alive without active disease; AWAD: Alive with active disease; DOD: Died of disease.

the T1-weighted sequences[9,19]; however, it is common that patients are taken care and followed up about these problems at the Endocrinology Unit until when Gd-enhanced MRI reveals a thickened pituitary stalk and/or a hypothalamic mass. Generally, it takes a year or longer for the mass to be biopsied and correct diagnosis be confirmed. Even when the diagnosis is confirmed, there are occasions that it takes time for the patient to be referred to hemato-oncologists for chemotherapy. Whenever the diagnosis and the introduction of treatment are delayed, the patient may develop not only endocrine problems but also cognitive impairment such as memory deficits as well as consciousness disturbances, as shown in our cases (Cases 12, 16, 17).

LCH in association with childbirth

The development of LCH in association with childbirth has not been well recognized. Regarding LCH occurring during pregnancy, only a few sporadic cases have been described previously[20,21]; however, no information is available how childbirth influenced on the development of LCH. In our series, the correlation between pregnancy/childbirth and LCH in adult female patients was noted in 4 cases (Cases 1-3, 12). Sex hormones are believed to participate in immune responses, as estrogens have been found to serve as enhancers in humoral immunity while androgens/progesterone appears to act as natural immune-suppressors[22]. For examples, postpartum thyroiditis/diabetes mellitus is speculated to be a consequence of the immunological flare that occurs after the lifting of the pregnancy-related immune suppression[23,24]. Moreover, pregnancy and the post-partum period are associated with increased breast cancer aggressiveness[25]. Thus, the hormonal imbalance in the postpartum period may trigger the development of LCH. Detailed examination of pregnancy and childbirth history in female LCH patients may clarify whether the associated hormonal changes influence the pathogenesis and the development of LCH.

LCH in association with various diseases/events

Two patients (Cases 16, 17) were noted to have cognitive disturbance due to LCH-related neurodegenerative CNS disease[10]. Additionally, two patients (Cases 15, 18) developed malignant lymphoma; one with concurrent LCH and Hodgkin disease and the other developed B-cell lymphoma after systemic chemotherapy for LCH. The association of LCH and other malignant lymphoid neoplasms has been well recognized[26-28]. In this series two patients (Cases 8, 9) with severe atopic dermatitis were found to develop LCH. This is an interesting topic considering the antigen-stimulation in the skin. It is also cautioned that recalcitrant or clinically atypical skin eruptions must be differentiated from LCH and other rare disorders[29] but no data is available that incidence of LCH is higher in patients with severe atopic dermatitis. Four patients (Cases 3, 5, 7, 8) were diagnosed to have LCH from spinal bone lesions. Particularly, of whom two had single spine (C3 or C6) involvement, not in the spinal body but in the arch. Spinal lesion should be searched for any adult who complained of cervical pain[30]. Intriguingly, discovery of LCH was triggered by road traffic accident in 2 patients (Cases 8, 17), although such reports are rarely found[31]. In Case 8, LCH lesion at the cervical spine was identified at the emergency hospital. In Case 17, traffic accident

incidentally led to the diagnosis of CNS- and pulmonary-LCH in the patient.

Importance of CT/MR/PET imaging for the diagnosis

To determine the precise biopsy/excision site of LCH, CT/MRI findings are inevitable. Particularly, bone scintigraphy for multiple bone lesions and Gd-enhanced MRI for CNS lesions are essential for the diagnosis of LCH[9,19]. However, more recently, ^{18}F-FDG PET is recommended. In one large study it was concluded that whole body FDG-PET scans can detect LCH activity and is useful to evaluate early response to therapy with greater accuracy than other imaging modalities (MRI, CT, plain films) in patients with LCH lesions in the bones and soft tissues[32]. Also, it is a useful tool for the monitoring of CNS disease activity in LCH[33,34]. It is said that ^{18}F-FDG PET might be useful to detect an early neurodegenerative lesions before MRI abnormalities appear, where bilateral hypometabolism is shown in the cerebellum and the basal ganglia (caudate nuclei) areas[34].

Therapeutic measures for adult LCH

In this case series, four patients received surgical resection of LCH mass without immuno-chemotherapy. Four patients received irradiation to the CNS-mass (n = 2), bone (n = 1) and lungs (n = 1), in association with immuno-chemotherapy. In the majority, systemic immuno-chemotherapy was given, mostly with a conventional combination of VBL/PSL or JCSL-96 protocol including VCR/cytosine arabinoside (AraC)/PSL[13] for induction. In 3 cases with CNS-LCH, 2CDA was employed. Previously proposed A1 protocol for adult LCH[6] was used only in one case in this series. With these measures, 6 ASAD cases were obtained, but necessity for further improvement of treatment for adult LCH seems apparent. As future trials, we have to scrutinize how efficiently we can employ AraC, 2CDA, clofarabine, and other novel agents for adult LCH patients. In the past, treatment reports on adult LCH cases were very limited[35,36]. In particular, the usefulness of intravenous 2CDA for CNS-LCH as well as for systemic MS-LCH was described in adult patients[37-40]. Windebank *et al*[41] also reported the usefulness of subcutaneous 2CDA treatment (5 mg/m^2 × 5 d, *sc*, q4 wk, for up to 6 cycles) in LCH. Effectiveness of the combination of 2CDA/AraC was described for extremely refractory cases[42]. More recently, effectiveness of clofarabine (25 mg/m^2 × 5 d, *iv*, q4 wk) has also been reported[43,44]. Particularly, Simko *et al*[44] demonstrated usefulness of clofarabine for multifocal skull lesions. On the other hand, Morimoto *et al*[11] reported the usefulness of Special C regimen of JLSG for treating adult LCH patients on ambulatory basis. Intriguingly, for the treatment of multiple bone LCH lesions in adults, Cantu *et al*[45] reported that AraC alone is an effective and minimally toxic, while VBL/PSL results in poor overall responses with excessive toxicity. Considering the fact that about 50% of LCH possess BRAF V600E mutation, molecular targetting treatment with vemurafenib has been proposed more recently[46]. As bone therapy regimen, Zoledronic acid as bisphosphonate is available, although its effectiveness on LCH bone lesions is still elusive[47]. Allogeneic HSCT for adult LCH is not within a scope of this article, although a few reports on pediatric LCH cases have been described[48,49]. As well recognized, in the recipients of allogeneic HSCT, care must be taken for the transplant related adverse events. In Table 2, a list of candidate systemic immuno-chemotherapy regimens is summarized, which we think is useful in choosing regimens for adult LCH patients. In practice, for an adult case of LCH with persistent minimal disease and systemic involvement, we prefer once a month or twice a month treatment, like Special C regimen of JLSG[11]. However, with these regimens, some adult patients may still show VBL neurotoxicity, MTX hepatotoxicity, or neutropenia due to mercatopurine hydrate (6MP); such events make it difficult to achieve the entire regimens as planned. Although we recognize that 2CDA is highly effective and could be useful in adult LCH, it is often difficult persuading the patients to stay in the hospital for the 5-d continuous treatment. If subcutaneous 2CDA is available at the outpatient care, this agent could be more employed in the treatment of adult LCH. In any case, it is important to make a most appropriate treatment plan for each patient individually. In summary, for adult patients with two major types of LCH, *i.e.*, recalcitrant multiple bone lesions and/or a mass at the HPR, early introduction of systemic immuno-chemotherapy using conventional regimens including AraC or alternative 2CDA or clofarabine regimens is recommended to overcome the disease-related impairment of qualify of life.

Table 2 Therapeutic options in the treatment of adult langerhans cell histiocytosis

Protocol	Drugs	Ref.
A1 protocol	VBL/PSL	[6]
JLSG-96	VCR/AraC/MTX/6MP/PSL	[13]
Cladribine-based	2CDA/PSL, 2CDA/AraC	[37-41]
Clofarabine-based	Clofarabine	[43,44]
JLSG-special C	VBL/MTX/6MP/PSL	[11]
Others	AraC alone	[45]
Molecular targetting	Vemurafenib	[46]
Bone therapy	Zoledronic acid	[47]

VBL: Vinblastine; PSL: Prednisolone; MTX: Methotrexate; 6MP: 6-mercatopurine hydrate; 2CDA: 2-deoxychloroadenosine.

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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