Nijmegen breakage syndrome. Diagnostic difficulties in primary immunodeficiency

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Abstract

Recurrent respiratory tract infections are one of the most common manifestations of primary immunodeficiencies in children. The diagnosis is often based on the results of broad differential diagnostic procedures. These should include not only evaluation of the immune system and identification of the infectious agents, but also clinical evaluation which should take into consideration variable manifestations from different organs and systems which together comprise the syndrome. The aim of this study is to present both the pathogenetic aspects of Nijmegen breakage syndrome, with particular emphasis on immunodeficiency and clinical manifestations and also of difficulties accompanying diagnostic procedures. The material of the paper consists of a presentation of the case of a nine-year old girl with Nijmegen breakage syndrome, manifesting symptoms of advanced respiratory disease. Diagnosis of the primary immunodeficiency syndrome requires the standards used in diagnosis and therapy of the immune system.

Key words: Nimegen breakage syndrome, immunodeficiency, children.

(Centr Eur J Immunol 2005; 30 (3-4): 83-88)

Introduction

Primary immunodeficiencies account for a large group of diseases often diagnosed too rarely and too late. Diagnosis of the underlying disease is frequently made on the basis of broad diagnostic procedures for recurrent respiratory tract infections. Immunodeficiency may be one of the elements of a heterogenous constellation of abnormalities from different organs and systems. Considering syndromic manifestations of the disease may be helpful in establishing the final diagnosis.

The aim of the following case report of a patient with Nijmegen breakage syndrome is to present both the pathogenetic aspects of this primary immunodeficiency syndrome, and also to demonstrate the considerable difficulties accompanying diagnostic procedures leading to the definitive diagnosis.

Representative case report

A nine-year-old girl presenting microcephaly and microsomia, with cystic fibrosis diagnosed six months earlier, was referred to the Department of Paediatric Pneumonology, Allergology and Clinical Immunology of the University of Medical Sciences in Poznań for the purpose of diagnostic procedures and establishing the principles of chronic treatment.

She is the third child of young non – consanguinous parents from the fifth pregnancy and natural delivery in the 36th week of gestation; her birth weight was 2100 g (value between the 3rd and 10th percentile). There is a lack of data from the neonatal period concerning body length and head circumference. The perinatal period was complicated by severe neonatal asphyxia (on the Apgar scale she was evaluated at 1-3 points in the 1st and 3rd minutes respectively) and symptoms of intrauterine infection.

The family history in respect of allergic diseases and chronic respiratory disorders is non-contributory; her older brother manifests microcephaly and mental retardation.

Since early infancy the girl has suffered from recurrent airway infections, presented a chronic cough, and persistent changes on auscultation in the lungs. Treatment was principally based on antibiotics and mucolytic agents;

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periodically, in exacerbations, bronchodilators, and systemic and inhaled corticosteroids were used as well.

At the age of nine, during hospitalization in the paediatric phthisiopneumonology ward, a diagnosis of cystic fibrosis was established with regard to the above mentioned personal history, bronchoscopy results revealing symptoms of chronic bronchitis, and chloride ion concentration in the sweat test: 75 mmol/l and twice 50 mmol/l. The diagnosis was not confirmed in the molecular analysis of thirty CFTR gene mutations. At that time high resolution computed tomography was carried out as well; the examination revealed sacculated bronchiectases located in the basilar segments of both lungs and in the right middle lobe, mainly in the fifth segment. In bronchial lavage bacterial pathogens were not cultured, nevertheless considering the presented general and respiratory symptoms – fever, cough, exercise intolerance, changes on auscultation in the lungs intensive antibiotic therapy was carried out. For the purpose of continuation of the therapy the girl was afterwards directed to the sanatory hospital where an infection with Pseudomonas aeruginosa was found in sputum culture.

The girl's psychomotor development in the first years of life was not substantially delayed, however, impaired speech and intellectual development were observed.

An early symptom was a proportional growth deceleration; the growth delay was corresponding with low body weight. At the age of six, microsomia was the reason for diagnostic hospitalisation in the paediatric endocrinology department. The results of the hormonal tests did not show any abnormalities.

On admission to our department the girl's general appearance was sufficiently well; she presented episodes of a tiring productive cough, retention of large amounts of thick discharge in the airways, and drumstick fingers. In a physical examination, both disseminated fine and coarse rales and crackles were found, as well as a bronchial sound on the front surface of the chest at the base of the right lung. Short stature and low body weight (110 cm and 15.8 kg, respectively – both values below the 3rd percentile) were noticed as well as microcephaly and dysmorphic face with sloping forehead, upward slant of the palpebral fissure, prominent mid – face with long nose, receding mandible and large auricles. On chest radiograph we found augmentation of the bronchial pattern in the central lung zones, mainly in the right middle lobe with accompanying hyperinflation mostly expressed in the lower lobes. Bilaterally, a total opacification of the maxillary sinuses was found in the radiographic examination. Spirometry revealed impaired ventilatory parameters of both obstructive and restrictive types.

Moreover, laboratory data of the inflammatory process (C-reactive protein, erythrocyte sedimentation rate, alpha1 – acid glycoprotein, and fibrinogen) were noticed. A sweat test using the pilocarpin ionthophoresis was carried out twice and revealed chloride ion concentrations within the normal range (41-43 mmol/l). Evaluation of the gastrointestinal tract, with ultrasonography of the abdominal cavity, and steatocrite in three days stool collection did not detect any abnormalities.

Taking into consideration inconsistent clinical manifestations, the results of the radiographic examinations (changes in the middle and lower portions of the lungs might be suggestive of ciliary pathology), current results of the sweat tests, and negative genetic examination of the cystic fibrosis gene mutations, bronchoscopy with bronchial biopsy for electron microscope examination of the mucous membrane was carried out. Endoscopy revealed the presence of a thick, mucopurulent discharge in the airway lumen, with congestion and swelling of the mucous membrane. However, a bronchial lavage culture did not show bacterial pathogens. In cytological examination of the bronchial secretion, dense protein masses with predominating neutrophils (75%) were found, as well as epithelial cells with cilia deprivation (15%), scarce macrophages, plasmocytes, lymphocytes and eosinophils. A chronic inflammatory process of the bronchial mucous membrane, with impaired regeneration of the epithelium, was found in the histological examination. With regard to the lack of ciliary cross – sections in the biopsy specimen, neither confirmation nor exclusion of the immotile cilia syndrome was able to be done.

The treatment consisted of intensive intravenous antibiotic therapy, mucolytic and bronchodilatatory agents, and inhaled corticosteroids as well as intensive chest physiotherapy. Taking into consideration the presence of persistent changes in auscultation, systemic corticosteroids, as well as an attempt of treatment with nebulized rh-DNase were introduced. As a result of such intensive multidirectional therapy, alleviation of cough episodes and of changes in lung auscultation as well as an improvement in ventilatory parameters in spirometry were achieved.

An extensive differential diagnosis of recurrent respiratory infections was carried out. We excluded: alpha1-antitrypsin deficiency, cardiovascular abnormalities, and taking into account a possible allergic background for the respiratory symptoms, total serum IgE concentration was examined, showing value within the normal range for this age. Evaluation of the immune system comprised examination of the main classes of immunoglobulin concentrations in serum – it revealed increased concentrations of IgM and IgA, yet IgG concentration was on the upper borderline of the normal value for the patient's age. Examination of IgG subclasses was not done because of the lack of a diagnostic method in that time. The concentration of specific postvaccination anti-HBs antibodies was 0.0 mIU/ml. Considerable abnormalities were evaluated in a cytometric analysis of the blood leukocyte immunophenotype; in the examined population of leukocytes the percentage of lymphocytes was 12; in their phenotype, a decrease in the absolute value and percentage of B cells (26% i.e. 200 cells/mm^3), T cells (48% i.e. 369 cells/mm^3), as well as the CD4 population (26% i.e. 200 cells/mm^3) and CD8 population (21% i.e. 161 cells/mm^3) was found. The relation CD4/CD8 was within the normal range and was 1.24. Evaluation of granulocyte function included the enzymatic

activity of neutrophils evaluated in the NBT test (it was on the lower borderline of the normal value), and a demonstration of full expression of the adhesive molecules CD11a, CD11b, CD11c and CD18. Serum concentrations of C3 and C4 complement components were normal.

On the base of the neurosurgeon's and geneticist's opinions suggesting a genetically determined autosomal recessive microcephaly, skull X-ray and karyotyping were carried out. On the neurologist's recommendation electroencephalography was performed, demonstrating abnormalities in the form of immature background activity and single episodes of hypersynchronic sharp waves in the frontal points, however, medication was not indicated.

The patient's psychomotor development was also evaluated. In the Terman-Merrill test the age of intellectual development was 5 years 8 months (real age 9 years 9 months); the intelligence quotient was 58 and was the equivalent of mental retardation in a mild degree. The girl manifested problems with concentration, difficulties with calculation and defining conceptions; her psychomotor skills were decreased, general development was unharmonious, and she showed emotional immaturity.

Taking into consideration the clinical manifestations, personal and family history in connection with the results of differential diagnosis, a suspicion of primary immunodeficiency syndrome was advanced and the need of a consultation in the Department of Gastroenterology, Hepatology and Clinical Immunology of the Children's Memorial Health Institute in Warsaw was arranged. The working clinical diagnosis of Nijmegen breakage syndrome was documented with a genetic examination; the SSCP analysis demonstrated homozygous nt deletion in the 657-661 position in the NBS1 gene (657del5), confirming the initial diagnosis in our patient. In her older brother, presenting microcephaly, mental retardation and recurrent respiratory infections, the NBS gene mutation was also detected and diagnosis of Nijmegen breakage syndrome was established.

After three months the girl was again admitted to our department with pneumonia, treated for one day in the regional hospital, where her state was progressively deteriorating. She developed severe dyspnoea, and aggravating episodes of tiring coughing with evacuation of large amounts of purulent sputum. The chest radiological examination was then indispensable despite of physicians' awareness concerning the radiosensitivity in NBS patients. It revealed extensive alveolar and interstitial inflammatory changes, most intensive in the central lung zones and in the lower lobes of both lungs. There were increased laboratory indices of inflammation, and serum concentrations of immunoglobulins considerably exceeding the normal range: IgG 2380-2310 mg/dl, IgA 223-350 mg/dl, IgM 604-728 mg/dl. Microbiology studies revealed Pseudomonas aeruginosa and Pseudomonas mucous type in the sputum specimen and Acinetobacter lwoffi in the blood culture. Infection with atypical microorganisms, such as Pneumocystis carinii, Mycoplasma pneumoniae, Chlamydia pneumoniae, Cytomegalovirus, Aspergillus and Mycobacterium tuberculosis was excluded. Despite intensive pharmacotherapy, neither clinical nor radiological improvement was achieved; the respiratory distress and oxygen dependence were intensifying and gasometric parameters of respiratory insufficiency were deteriorating. The therapy was continued in the Department of Anaesthesiology and Paediatric Intensive Care; for fourteen days mechanical ventilation, intensive antibiotic therapy and symptomatic treatment were administered. As a result of the therapy, a notable alleviation of the respiratory symptoms was achieved, but in radiological imaging there were still present strand densities in both lungs, with intensive alveolar emphysema particularly in the lower lobes, and marked interstitial changes.

After three weeks the girl again required hospitalization in our department because of respiratory insufficiency in the course of the next airway infection. A chest X-ray revealed considerable progress of the interstitial infiltrations and the presence of hyperlucent bullous changes in the right middle and left lower lobes. On the first day of hospitalization massive bleeding occurred from the respiratory tract. Computed tomography disclosed narrowing and modelling of the left lower lobar bronchus on a solid change present in the bifurcation of the lobar bronchi, which might be the equivalent of a conglomerate of lymph nodes. However, this CT scan presentation was not suggestive of any malignant process and no further diagnostic procedures to evaluate malignancy were needed. Furthermore, in both lungs, disseminated pneumatocele were demonstrated, particularly numerous in the middle lobe; also in this area, strand fibrous changes were visible. In all lobes the "white glass" image predominated, and there were numerous densities of atelectatic foci. In segments 1-2 and 3 of the left lung a "honeycomb" image with thickened bronchial walls was revealed. The whole radiographic presentation suggested an inflammatory process of the fine airways, bronchiolitis. In that time, after diagnosis of Nijmegen breakage syndrome was confirmed on a basis of the result of the genetic examination, accordingly to the recommendation of the Department of Gastroenerology, Hepatology and Clinical Immunology of the Chidren's Health Memorial Institute systematic at four week intervals programmed transfusions of immunoglobulins were started and preventive antibacterial and antifungal treatment was introduced. Since that time the girl has not suffered from as severe as previously exacerbations of the broncho – pulmonary disease in the course of respiratory infections. The patient requires regular monitoring and screening toward malignancy.

Discussion

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease classified in the group of chromosomal instability syndromes, together with Bloom's

syndrome, ataxia – teleangiectasia (Louis-Barr syndrome), Fanconi's anaemia and xeroderma pigmentosa. Spontaneous chromosomal instability, a predisposition to malignant processes, and immune deficiency characterise Nijmegen syndrome, Bloom's syndrome and ataxia – teleangiectasia. The mutated gene responsible for Nijmegen breakage syndrome, NBS1, is located on chromosome 8q21. The protein product of the gene, nibrin, is an element of the hMRE/hRAD50 complex, which is indispensable in the process of DNA double strand break repairs.

The syndrome was initially described in 1981 by Weemaes and co-workers from the University of Nijmegen in Holland; in 2000 the authors accomplished a clinical and laboratory analysis of all cases with NBS [1]. At that time there were 55 patients with NBS in the registry, among them 36 persons alive, the majority coming from eastern and central Europe, particularly of Polish origin.

The most constant clinical feature of the syndrome is microcephaly. In about 75% of patients the value of the head circumference is already below the 3rd percentile at birth. However, this means that microcephaly is not present in all neonates with NBS, although it is considerably expressed and is progressive in the first months of life. Growth retardation is an early manifestation – the body height of all NBS patients does not exceed the 10th percentile, and is proportional to the low body weight. In infancy, difficulties with feeding may occur, resulting from impaired development of the mandible. In the first years of life common problems are hyperreactivity and learning difficulties. Mental development is normal in about 40% of patients, the next 50% of patients are mildly retarded, and 10% are moderately retarded. Weemaes et al. did not demonstrate any correlation between the advance of microcephaly and the degree of mental retardation. In the first described patient with Nijmegen breakage syndrome microcephaly evaluated in the neuropathological examination was associated with diminished gyration of the cerebral cortex, particularly in the frontal lobes. A prominent structure demonstrated on the surface of the corpus callosum suggested incomplete development of the limbic system elements. A considerable reduction of cortical neurone suggests a role for the NBS1 gene in the development of the cerebral cortex [2].

A characteristic dysmorphic face with sloping forehead, a prominent mid-face with a long nose and prolonged filtrum, receding mandible, and an upward slant of the palpebral fissures with often present epicanthus are seen in all older patients, as well as dysplastic auricles and sparse hair.

Skin manifestations include: café au lait spots, vitiligo, and less commonly – teleangiectasiae, a palpebral hypersensitivity to sunlight and pigment deposition in the fundus of the eye.

In half of the NBS patients congenital abnormalities occur; among these are clinodactily and/or syndactily, anal atresia, ovarian dysgenesis, dysplasia of the hip; in particular patients there have also been hypoplastic trachea, renal hypoplasia and agenesia, retained testes, and abnormalities of the brain structures described [1]. Polish authors – Chrzanowska, Bekiesińska-Figatowska and Jóźwiak [3] have demonstrated hypoplasia of the corpus callosum in five out of seventeen children in a study group of patients with NBS.

The immune deficiency in Nijmegen breakage syndrome manifests as recurrent infections, both community-acquired and opportunistic, particularly involving the airways and urinary tract. Hypoglobulinaemia IgG and IgA as well as IgG2 and IgG4 subclasses deficiency, and defects in specific antibody production are characteristic laboratory findings [4]. In sixteen out of fifty five registered patients, Weemaes et al. demonstrated agammaglobulinaemia [1]. A detailed evaluation of humoral immunity in a group of forty Polish NBS patients was carried out by Gregorek et al. [5]. The authors paid particular attention to the considerable heterogeneity of the immune deficiency in Nijmegen syndrome, and its tendency to progress in time. In 80% of affected children a deficiency of one or more isotypes of immunoglobulin was demonstrated – the most commonly a combined IgG and IgA deficiency, subsequently, an isolated IgG deficiency, and next by rotation – a deficiency of all three main classes of immunoglobulins. Simultaneously, in five children a markedly elevated serum concentration of IgM was noted. The most characteristic feature of humoral immune defect present in all patients was a deficiency of at least one IgG subclass, and, most commonly, of all four subclasses. The evaluation of biosynthesis of specific antibodies revealed a low concentration of IgG antibodies to pneumococcal polysaccharide antigens and a defect in post-vaccine immunity in 75% of cases: production of only IgM anti-HBs antibodies or a total lack of response to a vaccination. Reports given by Reina-San-Martin et al. [6], Kracker et al. [7], and Xu et al. [8] have demonstrated a decrease of the class switch recombination (CSR) process in B lymph cells deficient of NBS protein. This defect of CSR is intrinsic, independent of transcription of the specific region, and results from uneffective recombination at the DNA level. The results of these studies suggest that NBS1 gene plays a crucial role in the process of immunoglobulin class switch recombination.

The most commonly assessed defect in cellular immunity manifests as a decrease in the percentage of CD3 lymphocytes and of CD4 subpopulation, leading to a diminished CD4/CD8 relation. The response of lymphocytes to a stimulation with a mitogen (phytohaemagglutinin) is decreased in the majority of patients. Polish investigators – Michałkiewicz et al. [9] made a considerable contribution to the evaluation of the immune system in Nijmegen breakage syndrome. They described in detail T lymph cell profiles in a group of thirty six patients, particularly highlighting the role of the NBS gene in the creation of TCR receptor. The authors demonstrated a profound defect in CD4 and CD8 subpopulations of T lymphocytes and a shift towards T memory cells (CD45RO), and displaying expression of γδ-TCR receptor, as well as an increase in NK cells

population (CD56). These data suggest a likely defect in regeneration of thymus – dependent T cells and a predominant role for the thymus – independent mechanisms of lymphocyte generation in Nijmegen breakage syndrome. Analogous abnormalities to those noticed by Michałkiewicz et al. have also been demonstrated by other investigators [10].

The cells of NBS patients are characterized by an efficient process of V(D)J recombination; however, they display an increased number of translocations involving the V(D)J locus [4]. The process of V(D)J recombination initiated by recombination activation genes RAG1 and RAG2 is associated with the phenomenon of precise DNA double strand breaks between the segments of genes coding for immunoglobulins and T cell receptors. In the process of recombination, the mechanisms signalling the damage to the DNA structure are simultaneously activated; among them, important components are γ-H2AX histone and a complex of the NBS protein with MRE11/RAD50 [11].

Among fifty five patients with NBS described by Weemaes et al., twenty two of them developed malignant processes, particularly B-cell lymphomas; in others leukaemias, central nervous system tumours (medulloblastoma, glioma) and rhabdomyosarcoma were diagnosed [1].

In contrast to ataxia teleangiectasia, the serum α-fetoprotein concentration in NBS remains unelevated.

Confirmation of the clinical diagnosis is achieved on the basis of genetic examination. The analysis of karyotype in NBS does not show abnormalities and plays a role in differentiation between this syndrome and chromosomal aberration syndromes. All NBS patients demonstrate cytogenetic aberrations in metaphasal lymphocytes after stimulation with phytohemagglutinin. In majority rearrangements – inversions and translocations occur in the 7^{th} and 14^{th} chromosomes and are localized in the positions of genes coding for immunoglobulin or T cell receptors. The most commonly reported aberration in Nijmegen breakage syndrome is inv(7)(p13q15).

An impaired response to ionizing radiation is a characteristic disorder of fibroblasts and lymph cells; this hypersensitivity to X-rays manifests as an increased number of chromosomal aberrations. Moreover, NBS patients' cells have a decreased ability to retain of the cycle or to slow down the S phase after exposure to high doses of ionizing radiation.

The definitive diagnosis of Nijmegen breakage syndrome may be assessed by demonstration of the NBS1 gene mutation – a deletion of five base pairs in the region 657-661 (657-661delACAAA), the mutation which was identified in our patient.

In the Slav populations – Polish, Czech and Ukrainian, epidemiological studies revealed an unexpectedly high prevalence of the above mentioned mutation of the NBS1 gene: 1/177 individuals, i.e. in about 0.56% of the population [12]. In comparison, in the German population the estimated frequency of the NBS1 gene mutation is much lower, being found in 1/866 individuals [13].

Investigations concerning the product of the affected gene, the NBS1 protein (nibrin, p95) provided information about its role in the repair of DNA double strand breaks caused by exposure to ionizing radiation. NBS1 is a crucial regulatory element in the protein complex RAD50/MRE11/NBS1 (R/M/N), which plays a role as a detector and mediator of the response to cellular DNA lesions. NBS1 augments the enzymatic activity of MRE11 and directs the R/M/N protein complex to to the place of DNA damage, where it forms nuclear conglomerations by interaction with phosphorilated histone protein H2AX. The R/M/N complex also activates ATM kinase – an enzyme significant in the activation of signalling mechanisms for DNA damage [13-19]. The impaired NBS protein synthesis leads to serious aberrations, resulting from the DNA double strand breaks, particularly in cells of high proliferative activity. This explains the presence of congenital abnormalities, immune deficiency, hypersensitivity to ionizing radiation, and a predisposition to malignancies.

Conclusions

Characteristic features of the phenotype in Nijmegen breakage syndrome, including microcephaly, may be an important premise, directing diagnostic procedures towards the primary immune deficiency. The case report of our patient described above points to the need for diagnostic examination of the immune system in every incidence of a chronic broncho-pulmonary disease in childhood. For these reasons elaboration of immune diagnostic standards, setting guidelines for definitive diagnosis, are indispensable. The importance of increasing knowledge about the syndrome and other primary immunodeficiencies should be stressed.

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