There are three main steps on the way to modern medicine in the Fundeni Pediatric Department, founded in 1959:
- 1959-1961: the Chief of the Clinic being Prof. Ion Nicolau MD, PhD, associated member of the Romanian Academy for Medical Sciences, who oriented the activity towards the field of pediatric Hematology and Oncology;
- 1961-1993, when the new Head of the Clinic - Prof. Gheorghe Goldis MD, PhD, member of the Romanian Academy for Medical Sciences - developed not only the Pediatric Hematology and Oncology, but also the Pediatric Nephrology, Rheumatology and Gastroenterology;
- since 1994 until the present days: during this period of time - under the leadership of Prof. Constantin Arion MD, PhD, member of the Romanian Academy for Medical Sciences - the activity was focused on the modern fields of contemporary interdisciplinary medicine (multi-modal treatment of cancer in children, hematological stem cell transplantation, hemodialysis and peritoneal dialysis, multi-modal treatment of connective tissue diseases, assistance for solid organ transplantation - kidney and liver, invasive procedures for liver exploration, treatment of portal hypertension in children …)

From the very beginning the staff was involved in the education and formation of students and of young specialists in Pediatrics, under the auspices of ‘Carol Davila’ University of Medicine and Pharmacy - the first (founded in 1857) and the most important School of Medicine in Romania. The educational duties interfered with the medical care offered to the sick children and teenagers, primarily to those suffering from cancer, non-malignant hematological diseases, kidney and urinary tract diseases, connective tissue diseases, congenital heart defects, chronic inflammatory disorders, chronic hepatitis and cirrhosis, haemofilias, thalassemias etc.).

The research activities were conducted to the same fields of modern medicine, Fundeni Clinic of Pediatrics having many priorities in Romania.

Last but not least, the doctors and nurses represented Romania at many national and international scientific meetings and participated in representative medical and scientific organisations.

Past and present medical and research priorities:

1. The 1959-1993 period:
1.1. Diagnose and treatment of pediatric hematological diseases, including hematological malignancies
- The foundation of the Hematological Laboratory of the Pediatric Department (dr. Lucia Lazar, dr. Stela Donovan, biologist Paraschiva Radamescu); the activity is now continued by dr. Mirela Asan and dr. Anca Gheorghe along with another two technicians;
- 1964: Prof. Gheorghe Goldis published the monography "Hemolytic Anemias" (Ed. Academiei, Bucuresti), the very first book dedicated to Pediatric Hematology in the romanian medical literature;
- In the 70's: the introduction of the techniques for thrombocyte functions evaluation and for the study of calitative platelet defects and von Willebrand's Disease in children (Dr. Caliopi Cutcuclache, Prof. Gh. Goldis);
- Also in the 60's and 70's: the first specialized approach to acute leukemias and lymphomas in children and teenagers, followed by many scientific papers and presentations at national and international congresses and symposia (Prof. Gh. Goldis, dr. Miriam Bercovici, dr. Ion Popescu et al)

1.2. Clinical activities and studies of the rheumatic and connective tissue diseases (dr. Ghita and colleagues); they published the monography 'Rheumatic Fever';

1.3. The introduction of modern diagnostic and treatment modalities for congenital heart defects, including the surgical approach in the Clinic of Cardiovascular Surgery, founded by Prof. Voinea Marinescu. His pioneer work was continued by prominent surgeons like Prof. Dan Setlacec, Prof. Marian Ionescu, Prof. Ioan Pop D. Popa, Prof. Dan Fagarasanu, by brilliant invasive investigationists like Dr. Bradu Fotiade and by the founder of the Romanian School of Anesthesiology and Intensive Care - Prof. G. Litarczeck;

1.4. The diagnose and treatment of urinary tract disorders in children, including urinary tract congenital anomalies, in co-operation with Fundeni Clinic of Urology (Prof. Gh. Olanescu, Prof. E. Proca, Prof. I. Sinescu and their collaborators)

1.5. Modern approaches to Staphylococcal respiratory tract infections and severe acute diarrheal disorders with important dehydration and to the parenteral nutrition in infants (dr. Valeriu Rosculet, dr. Ion Popescu);

1.6. The diagnose and treatment of portal hyper-tension in children (dr. Sanda Boiu, Dr. Tatiana Badea-luga). The surgical therapy was performed in many cases by Prof. Andrei Popovici and his collaborators from the Fundeni Department of Surgery;

1.7. The first kidney biopsies in children in Romania and and the first hemodialysis for chronic and acute renal failure in Romania On December 23rd, 1992, the first Pediatric Hemodialysis Unit in Romania was founded by dr. Gheorghe Chiриac-Babei;

1.8. The introduction of the multi-modal therapies for the solid tumors in children, in co-operation with the Fundeni Department of Surgery (Prof. Dan Setlacec, Prof. Andrei Popovici, Prof. Irinel Popescu, Assoc. Prof. Mihaela Ionescu and Assoc. Prof. Catalin Vasilescu), Fundeni Department of Urology (Prof. Eugen Proca, Prof. Ionel Sinescu), Fundeni Department of Radiology and Imaging (Prof. Cornel Butnaru, Prof. Serban Georgescu, Assoc. Prof. Constantin Zaharia, Assoc. Prof. Ioana Lupsescu, dr. Gheorghe Goldis Jr., dr. Mihai Lesaru) and Fundeni Department of Radiobioloy (Dr. Ioan Muntiu and colleagues).

2. The 1993 - 2005 period:

2.1. The extension of the Pediatric Dialysis Unit (which now has 5 full day operating machines, 6 days/week) and the introduction of new techniques for renal replacement therapy: Peritoneal Dialysis (a unit with 2 beds and 5 cyclers) and Hemofiltration. The Pediatric Dialysis Unit assists now 35 children with Chronic Renal Failure and performs 2800 hemodialysis procedures and over 18.000 peritoneal changes every year (Dr. Gheorghe Chiриac-Babei, Dr. Cristina Stoica, Dr. Mariana Vasilescu, Dr. Carmen Dinca, Dr. Bogdan Dima) - fig. 1 and 2

2.2. Co-operation for the first kidney transplantsations in children in Romania performed by Prof. Ionel Sinescu in the Fundeni Institute of Urology and Kidney Transplantation (1998 - from a living donor and 1999 with a cadaveric kidney)

2.3. Co-operation for the first liver transplantation in children in Romania, performed in 2000 by Prof. Irinel Popescu and his colleagues at the Fundeni Institute of General Surgery and Liver Transplantation;
2.4. The foundation in 1996 (dr. Ion Ivan) of the National Pilot Center for Thalassemia and Haemophyliac children and adolescents. The patients benefit from the modern diagnostic procedures (including hemoglobin electrophorese and molecular biology), from the substitution therapy and specific treatments (iron binding, the administration of coagulation factors concentrates etc); the unit co-operates in this field with Fundeni Department of Hematology (dr. Valentina Uscatescu, dr. Dan Coriu) - fig. 3.

2.5. The foundation of the Ambulatory Unit for Chemotherapy in children with malignancies (1997)

2.6. The foundation of the Bone Marrow Transplantation Department (2000) and the performing of the first pediatric autologous (March 2002) and allogenic (October 2003) hematopoietic stem cell transplantation for malignant disease in children in Romania. The team of doctors and nurses involved in HSCT in children is co-ordinated by Prof. Constantin Arion, Dr. Anca Colita, Dr. Luminta Dumitrache and the Chief-Nurse Rodica Ghelase. The program of pediatric stem cell transplantation is extended now to the solid tumors and the non-malignant hematological diseases (first allogenic BMT for severe aplastic anemia was performed in 2004); fig. 4, 5, 6.

2.7. Cardiac pathology for children is represented by Dr. Alin Nicolescu, cardiologist, that including diagnostic and treatment of cardiac malformations and rhythm disturbances, evaluation of children for transplant programs (renal, liver and bone marrow transplant).

2.8. Prof. Constantin Arion and Dr. Dorin Bleahu participated with chapters dedicated to Anemias and the Pathology of Leukocytes and Leukemias at the publishing of the most recent edition of the Romanian "Textbook of Pediatrics" (editors: Eugen...
Ciofu and Carmen Ciofu, Ed. Medicala, Bucuresti 2000)

2.9. Fundeni Clinic of Pediatrics got the confirmation as Center of Excellence in Hematology by CNCSIS - the National Authority for Research in Universities (2002)

We would like to underline the leading feature of activity in the Fundeni Clinic of Pediatrics: the constant preoccupation for co-operation with the most prominent Romanian and International Institutions; for the last category we mention prestigious Medical Centers like Medizinische Hochschule Hannover - Germany, L’Hopital ‘Armand Trousseau’ (Paris, France), L’Institut de Cancerologie Villejuif (France), Emory University (Atlanta, USA), Baylor College of Medicine (Houston, USA), Istituto Geanina Gaslini (Genova, Italia), The Jose Carreras Foundation, Regensburg University of Medicine (Germany).
THE LEFT VENTRICULAR DIASTOLIC FUNCTION AS PROGNOSTIC PREDICTOR IN PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY

Luminita Iliuta, H. Moldovan, D.P. Gherghiceanu, R.Vasile, Daniela Filipescu, B. Rădulescu, C. Macarie, V. Cândea

Institute of Cardiovascular Diseases ‘C.C.Iliescu’, Bucharest, Romania

Abstract Background: Previous studies have demonstrated that the presence of restrictive left ventricular (LV) diastolic filling pattern in patients with dilated cardiomyopathy have an unfavorable prognostic. Aim: 1. To establish the value of LV diastolic filling pattern as prognostic predictor in patients with idiopathic dilated cardiomyopathy. 2. Assessment of the betablocker treatment on these patients. Material and method: Prospective study on 143 patients (63% male, mean age 52 15) with dilated cardiomyopathy divided in 2 groups: 1. Group A - 87 patients with restrictive LV diastolic filling pattern, 2. Group B - 56 patients with nonrestricive LV diastolic filling pattern. 49 patients (56%) from group A and 31 patients (55%) from group B underwent betablocker treatment (carvedilol). Patients were evaluated every 3 months during a 2 year follow-up. Statistical analysis used SYSTAT and SPSS programs for the simple and multiple linear regression analysis, correlation coefficient and relative risk calculations. Results: 1. Mortality rate after 2 year follow-up was significantly higher in group A (68.96%) compared to group B (51.78%). The restrictive LV diastolic filling pattern (E wave deceleration time DT<130msec, E/A>2) has increased twice the risk of death (RR=2.6, p=0.007). 2. Clinical amelioration after 2 years was more frequent in patients with nonrestrictive diastolic filling pattern (DT>130msec, E/A<2). 3.Survival rate in patient undergoing betablocker treatment was similar in both groups (46.93% versus 51.61%) but significantly higher in these patients versus patients without betablocker treatment (46.93% versus 10.53% in group A, p=0.002, respectively 51.61% versus 44% in group B, p=001). 4. The LV diastolic pattern has remained restrictive at 2 year follow-up in 84.21% patients without betablocker treatment and only in 30.6% patients with betablocker treatment. Conclusions: 1. The persistence of the restrictive LV diastolic filling pattern at 2 years is associated with an increasing in the risk of death in patients with cardiac failure due to nonischemic dilated cardiomyopathy. 2. The association of restrictive LV diastolic filling pattern leads to a more unfavourable prognostic, with increasing the risk of death and worsening the clinical status of these patients in a 2 year follow-up. 3. The restrictive LV diastolic filling pattern is reversible on betablocker treatment.

Key words: dilatative cardiomyopathy, left ventricular diastolic filling pattern
Abbreviations: DCM - dilated cardiomyopathy, LV - left ventricle, LVEF- left ventricular ejection fraction, DT- deceleration time, NYHA - New York Heart Association

Address for correspondence: Dr. Luminita Iliuta, Institute of Cardiovascular Diseases ‘C.C.Iliescu’, Bucharest, Fundeni street 258, 022328, Bucharest, Romania
Introduction

Dilated cardiomyopathy is a significant cause of morbidity and mortality among patients with congestive heart failure and aging population. In the United States, the reported incidence of cardiomyopathies is 400,000-500,000 cases per year, with a prevalence of 2-3 million people [1].

In spite of modern therapy which associates vasodilators, angiotensin-converting enzyme inhibitors to digitalis treatment, and of progresses on surgical treatment, the overall prognostic remains poor. Facing this heart condition, clinician is in the position to decide which of various parameters should be used in order to evaluate the severity and the prognostic of disease.

The long and medium term prognosis in patients with idiopathic dilated cardiomyopathy is influenced by many parameters among which left ventricular diastolic function is one of the most important. It is also appears to be the one of the earliest detectable abnormalities in many of the heart disorders.

Diastolic dysfunction is a condition in which filling of the left ventricle is impeded, leading to symptoms of low cardiac output and/or elevated pulmonary venous pressure. There are two distinct abnormal filling patterns which can be detected by Doppler echocardiography [2]: 'impaired relaxation' characterized by a prolonged isovolumetric relaxation time, an increased deceleration time of early transmitral filling velocity (E wave) and a decrease in E/A ratio and 'restrictive pattern' characterized by a shortened isovolumetric relaxation time, a decreased deceleration time, and an elevated E/A ratio.

It is known that the presence of a restrictive left ventricular diastolic filling pattern is associated with a more unfavourable prognosis in most of cardiac diseases (valvular, coronary or congenital). The impact of restrictive left ventricular diastolic filling pattern presence on the evolution and prognosis in patients with idiopathic dilated cardiomyopathy has been evaluated in some previous studies and we tried to evaluate it also taking into consideration the beta-blocker treatment in these patients as well.

That is why the purpose of this study is to establish the implication of the left ventricular diastolic filling pattern on the evolution and prognostic in patients with idiopathic dilated cardiomyopathy. We have also tried to assess the impact of betablocker treatment on the left ventricular diastolic filling pattern in these patients.

Material and Methods

We carried out a prospective study on 143 patients with idiopathic dilated cardiomyopathy admitted to the Institute of the Cardiovascular Diseases ‘CC Iliescu’ between 1 January 2000 and 1 January 2003. Most of the patients were male (62.72%), with a mean age of 52±15 years and the mean left ventricular ejection fraction was 25±5.2%.

The patients were evaluated clinically and by echocardiography at the enrollment into study and during the treatment at every 3 months for two years. For each patient taken into study we assessed echocardiographically the left ventricular systolic and diastolic performance and the left atrium function.

All patients received the standard treatment for heart failure with digitalis, diuretics, converting enzyme inhibitors and spironolactone. At the enrollment into study all the patients were in sinus rhythm.

The left ventricular diastolic filling was evaluated by Doppler examination and the restrictive diastolic filling pattern was defined as an E wave deceleration time less than 130msec and the E wave /A wave velocity ratio more than 2.

Depending on the LV diastolic filling pattern the patients were divided in two groups (fig.1):

a) Group A - 87 patients with a left ventricular restrictive diastolic filling pattern, and
b) Group B - 56 patients with a left ventricular non restrictive filling pattern.

Depending on both diastolic performance of the left ventricle and the type of the treatment, each group was divided in two subgroups as follows:

a) Subgroup A1 with 49 patients with a restrictive LV diastolic filling pattern undergoing betablocker treatment;
b) Subgroup A2 - 38 patients with a restrictive diastolic filling pattern without betablocker treatment

c) Subgroup B1 comprising 31 patients with
an non restrictive LV filling pattern who underwent betablocker treatment, and

The two groups were comparable concerning: mean age, gender, mean LVEF, secondary mitral regurgitation degree, the mean pulmonary artery pressure.

Statistical analysis used SYSTAT and SPSS programs for the simple and multiple linear regression analysis, correlation coefficient calculation and relative risk calculation. The most important endpoints used for the estimation of the medium term prognosis were: type of LV diastolic filling pattern, NYHA class for heart failure, quality of life (appreciated on a scale from 1 to 10 using a questionnaire filled in by the patient at each visit), death.

Results

1. Mortality rate at 2 year follow-up was significantly higher in patients with restrictive LV diastolic filling pattern (68.96% in Group A) compared to patients with nonrestrictive LV diastolic filling pattern (51.78% in Group B), regardless the LV systolic performance.

2. The presence of the restrictive LV diastolic filling pattern (E wave DT<130msec, E/A>2) has increased the risk of death by 2.8 times at 1 year and by 2.6 times at 2 year follow up, regardless

the presence of other parameters known to increase mortality in patients with dilated cardiomyopathy. The restrictive left ventricular diastolic filling pattern turned out to be an independent predictor for increasing the risk of death or hospitalization for heart failure decompensations (p=0.001), regardless the left ventricle dimensions or performance, the presence of a secondary mitral regurgitation haemodinamically significant or pulmonary hypertension.

These data are presented in figure 2 which shows the relative risks of death at one year follow-up for the patients with dilative cardiomyopathy associated with different parameters known for increasing the mortality rate. Thus, the risk of death at one year was increased by 4.2 fold by the presence of rhythm disorders, by 2.8 fold by an associated restrictive left ventricular diastolic filling pattern, by 3.2 fold by a severe left ventricular systolic dysfunction with an ejection fraction less than 20% and by 1.9 fold by the presence of an associated haemodinamically significant mitral or tricuspid regurgitation.

In figure 3 there are represented the relative risks of death at two years follow-up for the patients taken into study associated with different known parameters that increase the mortality level in idiopathic dilated cardiomyopathy (such as the presence of atrial or ventricular rhythm disorders, an associated haemodinamically significant mitral or tricuspid regurgitation, systolic performance of left ventricle). The relative risks are also represented distinctly depending on the type of left ventricular diastolic filling pattern. The predictive value for
death at two years follow-up of the left ventricle systolic dysfunction, of the atrioventricular valve regurgitation or of the arrhythmias was higher in patients with a non-restrictive LV diastolic filling pattern. In these patients, values of left ventricular ejection fraction less than 20%, the presence of a mitral or tricuspid regurgitation three or four degree or ventricular arrhythmias increased about four times the risk for death at two years follow-up.

The presence of a restrictive left ventricular filling pattern homogenized the relative risk values. In patients from group A, the risk for two years mortality was increased by the type of the diastolic filling regardless the left ventricular systolic performance or the presence of mitral or tricuspid regurgitation or atrial arrhythmias. In these patients, the only independent predictor for increasing the risk of death at two years follow-up was the presence of ventricular rhythm disorders.

3. Taking into consideration the patient evolution, the percent of those with a favourable evolution quantified as NYHA class of heart failure less than 3 and the quality of life score >5 was higher in the group of patients with a non-restrictive LV diastolic filling pattern, regardless whether they received beta-blocker treatment or not.

Thus, at one year follow-up, the percent of patients in a low NYHA class was nearly double in group B and at two years follow-up three times higher compared with patients with a restrictive left ventricular diastolic filling pattern (group A), regardless the beta-blocker treatment undergone. In addition, the quality of life score >5 was found at about three fold more patients with a nonrestrictive LV diastolic filling compared with a restrictive one in both years of follow-up (fig. 4).

4. Follow-up at 2 years showed that survival rate in patients undergone beta-blocker treatment was similar in both types of the left ventricle diastolic filling pattern but significantly higher compared with patients without betablocker treatment:
   - In Group A 46.93% patients with betablocker treatment respectively 10.53% in patients without betablocker treatment, p=0.0022;
   - In Group B 51.61% patients with betablocker treatment respectively 44% in patients without betablocker treatment, p=0.001.

In figure 5 the percents of survivors at one year and two years follow-up are presented for all four subgroups taken into study depending on both the type of left ventricle diastolic filling and on the type of treatment (patients who received or not beta blockers):
   - In group A (patients with a restrictive left ventricular filling pattern) the survival rate at one year was approximately 2.5 times higher in patients who received betablocker treatment compared with those without betablocker treatment (about 66% versus 28%). In addition, at two years follow-up the survival rate in subgroup A1 was 3 times higher in patients with betablocker treatment (about 44% versus 14%);
   - In group B (patients with a non restrictive

![Fig. 3 - Risk of death at 2 years in patients with idiopathic dilated cardiomyopathy depending on the type of LV diastolic filling pattern](image-url)
left ventricular filling pattern) the percent of survivors at one year and at two years was approximately 1.5 times higher in patients with betablocker treatment compared with patients who did not receive this treatment.

5. Betablocker treatment has influenced the LV diastolic filling pattern evolution, too. Thus, LV diastolic filling pattern has remained restrictive at two years follow-up in 84.21% patients without betablocker treatment and only in 30.6% patients undergoing betablocker treatment. The evolution of the left ventricular diastolic filling was influenced by the betablockers. Thus, at two year follow-up, left ventricular diastolic filling pattern
has remained restrictive in about 84% patients who did not received betablockers and only in aproximatively one third - thirty percent of the patients undergoing betablocker treatment.

Discussions

Data from present study support the hypothesis tested in other previous studies which highlighted the importance of LV diastolic filling as predictor of severity and prognosis in dilated cardiomyopathy. 

Dilated cardiomyopathy is characterized by an abnormal LV diastolic filling and severe cases showed the restrictive type of diastolic pattern [19] with a E/A>2 cases with a poor prognosis and cases with a E wave DT<150ms usually indicating bad outcome.

The literature showed also that the restrictive LV diastolic filling pattern is frequent in dilated cardiomyopathy and is associated with more severe disease being the best predictor for cardiac death in these patients [6, 8, 10, 13, 15].

The mortality rates showed in our study are in line with those from literature. Thus, the mortality rate at 2 year follow-up found was of 68.96% in patients with dilated cardiomyopathy with a restrictive filling pattern and of 51.78% for those with a nonrestrictive filling pattern.

The clinical evaluation by NYHA class of heart failure and quality of life has shown an amelioration at 2 years which was more frequent in patients with nonrestrictive diastolic filling pattern, which is in line with the literature studied [5, 7, 8, 10, 11].

The survival rates presented by literature in patients with dilated cardiomyopathy have shown different figures. The survival rates at 2 year follow-up was 52% for patients with restrictive LV diastolic filling as compared with 94% in patients with a nonrestrictive filling pattern (defined as prolonged DT) [4]. Another study has shown a survival rate with standard therapy of 84%, 73% and 61% at 1, 2 and 4 years respectively that is significantly poorer than that of age- and gender-matched population.[9].

In our study the survival rates were calculated separately for the patients who underwent betablocker treatment or not. The figures at the 2 year follow-up for those who received standard treatment seems to be slightly lower than in the literature maybe because of the transplantation surgery which is not very well developed yet.

Previous studies have demonstrated that beta-adrenergic blocking therapy has a consistent beneficial effect on LV ejection fraction in cardiomyopathy patients [24] with a more variable and controversial effect on diastolic relaxation properties [20, 22, 23, 24, 25]. There was initially reported [23] an improvement in LV end-diastolic pressure with metoprolol. Subsequently, the MDC Study Group evaluated LV diastolic filling using transitmitral Doppler echocardiography [20]. They reported that metoprolol resulted in a significant improvement in early LV diastolic deceleration times in cardiomyopathy patients. The maximum improvement in deceleration times occurred within 3 months of initiation of betablocker therapy. These observations in a large group of cardiomyopathy patients are consistent with an improvement in diastolic relaxation properties [21].

These was shown by our study in which the betablocker treatment associated to the standard therapy has influenced the diastolic filling pattern which remained restrictive at 2 year follow-up in 84.21% patients without betablocker treatment and only in 30.6% patients with betablocker treatment.

Conclusions

In patients with dilated cardiomyopathy, the presence of a restrictive left ventricular diastolic filling pattern is associated to a more unfavorable prognosis. This type of filling increased the risk of death and worsened the clinical status of the patients (quantified as NYHA class and the quality of life).

On medium term, the use of betablockers associated with conventional therapy of heart failure was shown to improve the left ventricle diastolic filling and, in the same time to decrease the mortality rate and to improve the quality of life.

References

The Left Ventricular Diastolic Function as Prognostic Predictor in Patients with Diopathic Dilated Cardiomyopathy

Am Heart J 1991; 11: 951-957
MICROBIOLOGIC ANALYSIS OF A CEFEPIME; PRODUCING ISOLATES 1 - YEAR PERIOD

Jeni Laura Vlad

Department of Microbiology, Clinical Institute Fundeni

Abstract

Study objective. We have studied the Cefepime’s evolution, during a period of 1 year. Design. Review study. Setting. 106 patients have confirmed in culture with: Pseudomonas aeruginosa, Enterobacter spp., Proteus spp., Escherichia coli, Acinetobacter spp. and other non-fermentative Gram negative germs. Measurements and Main Results. Cefepime is a 4-rd generation cephalosporine. It has a unique structure at physiological pH. It has the advantage of an unique action in the empirical therapy. Since February 2004 to February 2005 in one study performed with 106 selected patients in Department of Microbiology of our Clinical Institute, have estimated in vitro action of this substance. The neutropenic patients have monitored particularly: 87% from isolates (75) was microbiologic reactive (sensible) and 13%(31) resistant. Conclusion. Cefepime rests a choice in severe infections (intra-abdominal infections, peritonitis, post-surgical infections, etc) with fermentative and non-fermentative aerobe and anaerobe Gram negative rods and also Gram positive germs. Only cefepime or in conjunction with other substances, increases the rate of clinical recovery. The patient’s compliance for selected antibacterial agents have a reduced frequency of a daily administration and when the cure is reduced (1-2g/12h, 7-10 days). Cefepime is comparable with carbapenemes. Cefepime is useful in the treatmnet of mixed infections and in the empirical treatment before involved germ identification.

Key words: Cefepim, bacteria, Cephalosporine IV, neutropenia, intraabdominal infection

Introduction

"Everything can be invented has been invented" Charles H. Duell, Commissioner, U.S. Office of Patents, 1899.

The bacteriological image against Cefepime hydrochloride represents the object of this microbiological study. We have estimated the substance’s action during the contact with the bacterium, which caused the illness. Thus, Cefepime is a 4-rd generation cephalosporine, with a complex chemical structure and a bactericidal effect.

The study has included the group of fermentative and non-fermentative, Gram negative bacteria.

The zwitterion structure confers hydrosolubility and fast penetration of bacterial membrane. The cefem-core the N-methyl pyrolidone group...
and the sin-oximinothiazole-chain, increase the activity of substance against Gram-negative fermentative and non fermentative rods and Gram positive germs.

These components also determine increased activity against Pseudomonas aeruginosa. The Cefepime has low activity with BUSH 1 group β lactamases (table 2); It penetrates the pores of bacterial membrane fastly. It has bactericidal effect in periolasmic space by increasing of concentration. It has high affinity for PBP (penicillin binding proteins); for exemple PBP 1, PBP 2, PBP 3 in E.coli, PBP 1, PBP 3 in Pseudomonas aeruginosa.
Material and method


_Bacterial culture_: Over 106 bacterial strains have tested with Cefepime in the Research Laboratory of Microbiology in our clinic.

_Culture media used for isolation_: Nutrient Agar-blood, Nutrient-Broth, Cled Agar, Mac Conkey Agar and Mueller Hinton Agar.

_Biochemical media for identification_: SIM (mobility-indole-hydrogen sulfide), TSI agar (Triple sugar iron agar), Urea Agar Base, Simmons Citrate Agar, Phenylalanine agar, Lysine iron agar.

_Pathological collections (samples):_ ascitic fluid, cholecystic fluid, drainage tubes, abscesses, various collections, blood, urine, bronchoalveolar aspirates, respiratory secretions.

_Testing conditions:_
- _Culture media:_ Mueller Hinton Broth with adapted cathion (CAMHB), Mueller Hinton Agar dilutions.
- _Inoculum:_ growth method or direct method with suspended colonies (0.5 McFarland standard).
- _Incubation:_ 35°C, 16-24 h, enviromental air;
- _Biodisks (30 mcg)_ and E-test strips with cefepime;
- _Same panels and the Phoenix 100 System (Becton Dickinson) were used for identification of bacterial species and for standard antibiogramme.

The Bactec System (BD) was used for screening detection of bacterial strains in hemoculture.

The result interpretation was performed according to the NCCLS Standard. (Table 3)

Interpretation should be as stated above for results using dilution techniques. Interpretation involves correlation of the diameter obtained in the disk test with the MIC for Cefepim (Fig. B).

The susceptibility to Cefepim can be determined by standardized test methods. The MIC values obtained should be interpreted according to the NCCLS standard.

Interpretation is identical to that stated above for result dilution techniques. As with other susceptibility techniques, the use of laboratory control microorganisms is required to control the technical aspects of the laboratory standardized procedures.

Cefepime is comparable to piperacillin-tazobactam and carbapenems. Therefore, cefepime, piperacillin-tazobactam, meropenem, imipenem should be selected for initial empiric antibiotic therapy. Same studies evaluate cefepim in mono-therapy comparable with ticarcillin/clavulanic acid with aztreonam in neutropenic patients.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Fast penetration</th>
<th>Low affinity for chromosome encoded β lactamases</th>
<th>High stability with β lactamases</th>
<th>High affinity for target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxipime</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| Table 2. The Biochemical action of Cefepime |

| Table 3. Interpretation of results. NCCLS (Table Enterobacteriaceae M7-mic) |
|-------------------------------|-----------------|-----------------|-----------------|
| **Interpretation of results** | **Sensible** | **Intermediate** | **Resistant** |
| Diffusion diameter (mm)     | >18mm           | 15-17mm         | <14mm           |
| Standard MIC(μg/ml)         | <8 μg/ml        | 16μg/ml         | >32 μg/ml       |

| Table 4. First therapy in neutropenic patient |
|-------------------------------|-----------------|-----------------|
| **Monotherapy** | **Combination** |
| Cefazidime    | Acylaminopenicilline + AMG |
| Cefepime      | Cefalosporin III/IV + AMG |
| Piperacillin-Tazobactam | Cefepime +Metronidazol* |
| Carbapenems   |                  |

J. L. Vlad
Microbiologic analysis of a Cefepime-producing isolates 1-year period.

**Fig. B - Diffusion diameter / E-test - MIC**

**Fig. C - Empiric initial therapy: IDSA guidelines**

- Fever > 38.3°C + Neutropenia < 500/μl
  - Low risk
    - Oral
    - quinolone + amoxicillin-clavulanic acid
    - cefepime
    - ceftazidime
    - carbapenem
  - High risk
    - I.V.
    - glycopeptide
    - monotherapy
    - aminoglycoside + cefepime
    - anti-pseudomonas β-lactam
    - ceftazidime + carbapenem ± aminoglycoside
Results

87% from tested strains were sensible with cefepime. Bacterial resistance was approximately 13%. Cefepime is a bactericidal agent with broad spectrum of action, useful in empirical therapy of various infections with parenteral treatment, which was evaluated in our hospital practice. Its efficiency was demonstrated particularly in intraabdominal mono or multibacterial infections.

It could be also indicated in the primary testing for a diversity of species of Pseudomonas spp. (other than P. aeruginosa), Stenotrophomonas maltophilia and Acinetobacter spp., (over 81% action) and particularly in neutropenic patients. Table 5, 6.

Conclusions

The clinical action was demonstrated be favorable in the next diagnosis: lower respiratory tract infections, urinary tract infections, skin infections, intraabdominal infections (peritonitis, cholecystitis, pancreatitis), sepsis, neutropenic patients, etc.

Cefepime is useful in treatment of mixed infections and also in empirical therapy before identification of causative germs.

The rate of bacterial response of the substance could be seen in the table 6.

Bacteriological response was determined to be eradication (presumed eradication/ causative-organism, absent or no material to culture in a patient who was clinically cured), persistence/ presumed persistence (causative organism present or no material to culture in a patient whose clinical response was failure), or indeterminate.

Cefepime could be only solution for empirical therapy. The association of cefepime with aminoglycosides was increased the sensitivity of strains. The association with metronidazole could be an efficient therapy in -patients with severe intraabdominal infections.

The in vivo and in vitro actions of the substance were approximately identical.

Cefepime rests a choice in severe infection (intra-abdominal infections, peritonitis, post-surgical infections, etc) with Gram negative fermentative or non-fermentative, aerobe and anaerobe rods and also Gram positive germs. Only Cefepime or in combination with other substances increases the rate of clinical recovery and is a revolution in the modern cephalosporins evolution.

Acknowledgement

Thanks to the members of Medical Department of Bristol Myers Squibb Romania for their ideas, suggestions and data.
References

NEEDLE CORE BIOPSY VERSUS NEEDLE ASPIRATION CYTOLOGY IN PREOPERATIVE DIAGNOSIS OF SOLID BREAST LESIONS

Mihaela Mihai\textsuperscript{1,3}, M. Lesaru\textsuperscript{2,4}, M. Ceausu\textsuperscript{3,4}, Carmen Ardeleanu\textsuperscript{3,4}

\textsuperscript{1}Department of Pathology, Fundeni Clinical Institute
\textsuperscript{2}Clinic of Radiology and Medical Imaging, Fundeni Clinical Institute
\textsuperscript{3}Victor Babes' National Institute of Pathology
\textsuperscript{4}Carol Davila' University of Medicine and Pharmacy

Abstract

Aim: Comparative study of the histological and cytological diagnosis value of specimens and samples obtained by needle core biopsy followed by aspiration in solid breast lesions. Material and methods: We performed ultrasound-guided needle core biopsy followed by aspiration for evaluation of 41 BI-RADS mammographic categories 2 (typical benign), 3 (probably benign), 4 (suspect) and 5 (probably malignant) breast lesions. After the preparation of the biological material obtained by aspiration the findings of the smears was compared with those of histological sections obtained by needle core biopsy and paraffin embedded. The results were interpreted and reported as C1-C5 and B1-B5 categories according to recommendations of European Commission Working Group for Breast Screening Pathology/European Breast Screening Program (2000). Results and discussions: The sensitivity, accuracy and negative predictive value of the histological diagnosis of tissue fragments obtained by needle core biopsy were proved to be superior those obtained by cytological examination of needle aspiration ones, proportion of false negative results in case of cellular aspirates being higher (5.72\%) than in microbiopsies (2.86\%). Concordance between breast imaging findings and morphological aspects was established in 37 cases and discordance was established in 4 cases. Conclusions: We consider that histological report of needle core biopsy specimens is a more accurate method evaluating breast lesions than the cytological report of the needle aspiration samples. Our study confirm the present day accepted opinion that needle core biopsy is characterized by performance indicators and diagnostic advantages better than needle aspiration cytology and is the standard method of assessment of preoperative solid breast lesions with undetermined or suspect clinical and imaging signification.

Key words: needle core breast biopsy, needle aspiration breast cytology, image guidance, solid breast lesions

Address for correspondence: Dr. Mihaela Mihai, Department of Pathology, Fundeni Clinical Institute, Fundeni street 258, 022328, Bucharest, Romania; E-mail: mictekro@yahoo.com
Introduction

Screening mammography performed regularly to female patients over 40 years with no symptoms can lead to an early identification of malignant lesions and also of many breast lesions with undetermined clinical and imaging signification [15, 20, 49]. In the last years huge efforts have been done for development of non-invasive or minimal invasive preoperative diagnostic methods because many of identified and radiologic characterized lesions as suspect for malignancy were proved to be benign after surgical treatment [11, 20, 26].

At the time needle core biopsy (tissue micro-biopsy) and fine needle aspiration cytology represent usual preoperative diagnostic methods in order to differentiate malignant form benign breast lesions [7, 20, 26, 45, 55]. Preoperative cytological and histological diagnosis of clinical suspect breast lesions allows the assessment of therapy and discussion with the patient decreasing in this way the number of useless surgical operations [11, 20, 26, 45, 55]. Despite of its simplicity fine needle aspiration cytology, which assumes to obtain biological material for cytology, is not an accessible method to any institution because it requires trained cytopathologists [11, 45], capable of interpreting the microscopic findings of the aspirate establishing a cytological diagnosis which does not make distinction between in situ lesions and invasive carcinomas and only occasional allows a tissue specific diagnosis for a benign lesion [11, 55].

Needle core biopsy (NCB), which assumes to obtain histological material (tissue samples), is characterized by superior performance indicators than fine needle aspiration cytology and in the last time has become the dominant method of preoperative diagnosis of palpable and non-palpable breast lesions [7, 11, 20, 45, 46, 55]. However, the superiority of one diagnostic method over the other has not yet been established, both of them being dependent on the nature of the breast lesion, the skill of the individual obtaining the sample and the skill of the pathologist interpreting the specimens [5, 46, 55].

In this study we tried to compare the diagnostic value of histological and cytological examination of tissue samples and aspirates obtained by ultrasound-guided needle core breast biopsy followed by aspiration in solid breast lesions, according to the recommendations of European Commission Working Group for Breast Screening Pathology / European Breast Screening Program (2000).

Material and Methods

Our study included 36 patients with solid breast lesions mammographically characterized as being suspects and probably malignant (BI-RADS 4 and 5 categories) and also 3 patients with mammographic abnormalities most probably benign (BI-RADS 3 categories); other 2 patients with benign abnormali-ties (BI-RADS 2 categories) were biopsied on demand. Ages of patients were between 30 and 73 years and lesions were clinically palpable and non-palpable. The cytohistopathological specimens were obtained under ultrasonographic guidance (Fig. 1) with True-Cut needles 14-18G (Cook, Bard) using a coaxial system (automated biopsy guns); after the tissue sampling an aspiration on the needle sheath has been done in order to perform cytological examination. The procedure required local anesthesia with 1% xilin and took 30-45 minutes per each case.

Three to nine 4 to 15 mm length tissue fragments were achieved per case. Samples were fixed in 10% buffered formalin and embedded in paraffin. Serial sections were cut at 4 m, one being stained with hematoxylin and eosin (H E) for routine histological and others for immunohistochemical assessments. The histological diagnosis followed the recommended criteria of European Commission Working Group for Breast Screening Pathology/European Breast Screening Program (2000) reporting every case as B1-B5 categories. Immunohistochemical tests have been done in some cases of positive biopsies for assessment of prognostic molecular factors. The biological material aspirated at the end of the procedure was quickly displayed on slides (3-12 preparations per case), dried in the air, fixed by immersion in methylc alcohol and stained with Giemsa. Results were reported as C1-C5 categories, according to recommendations of European Commission Working Group for Breast Screening Pathology/ European Breast Screening Program (2000). Immunohistochemical tests were
carried out on smears assessed as being cellular rich, but the results were not satisfactory compared with those done on histological sections of the tissue fragments. For sensitivity, specificity, predictive value calculus a descriptive statistic analysis has been done (binary tests).

Results

Two cases of mammographical abnormalities typical benign (BI-RADS 2 categories) were confirmed by histopathology and cytology (B2 and C2 categories), being interpreted as fibroadenoma and fibrocystic changes.

Among 3 cases mammographically characterized as most probably benign (BI-RADS 3 categories), one case with suspect cytology (C4 category) was histological confirmed (B5 category). The other 2 cases imaging assessed as being BI-RADS 3 abnormalities have been proved to be benign (1 case B2/C2 category and 1 case B3/C3 category) (Fig. 2); histopathology examination of the excision biopsies confirmed the benign nature of these specimens (fibroadenoma and sclerosing adenosis).

In our archive 10 cases were mammographically assessed as suspect abnormalities (BI-RADS 4 categories). 7 cases were reported as B5 category (positive biopsies), from which 6 cases with malignant cytology (C5 categories) and 1 case with suspect cytology (C4 category). One case has been microscopically assessed as benign lesion with unknown biological potential (B3 category) and cytological as benign lesion (C2 category); however the lesion proved to be malignant (invasive carcinoma) after the excision biopsy. Other 2 cases have been histological and cytological diagnosed as benign lesions (B2/C2 categories), such as isolated fibrosis, and the lesions were clinical and imaging stationary at 3 and 6 months interval.

The 26 cases mammographically assessed as being most probably malignant (BI-RADS 5 categories) were confirmed by histopathology examination: B5 categories (positive biopsies) - 26 cases, from which 23 cases with malignant cytology (C5 categories), 2 cases with suspect cytology (C4 categories) and 1 case with atypical cytology, probably benign (C3 category) (Fig. 3 and 4) (Table 1).

Diagnostic accuracy, sensitivity and negative predictive value of histological examination of tissue fragments obtained by needle core biopsy proved to be superior to those obtained by cytological examination of aspiration specimens, the proportion of false negative results in case of aspiration...
Needle core biopsy versus needle aspiration cytology in preoperative diagnosis of solid breast lesions

Fig. 2 - Benign solid breast lesion (same case: S.E., 40 years old): a. Benign cytology (Giemsa x 200); b. Fibroadenoma findings on the core biopsy (HE x 100)

Fig. 3 - Invasive breast carcinoma (same case: C.F., 40 years old): a. Negative cytology (C3 category, Giemsa x 400); b. Positive biopsy (B5b category, HEx 40)

Fig. 4 - Invasive ductal breast carcinoma with extensive intraductal component (same case: P.C., 56 years old): a. Positive cytology (C5 category, Giemsa x 400); b. Positive biopsy (B5a+b category, HEx 200)
samples being higher than those of microbiopsies (Table 2).

Concordant results between clinical-imaging and cyto-histological findings have been established in 37 cases. In 4 cases of discordant results was necessary imaging follow-up at short interval (2 cases) and excision biopsy (2 cases).

**Discussions**

Indicated by existing therapeutic guides and the international consensus [20] percutaneous breast microbiopsy with 14G devices is considered at this time the main diagnostic modality prior to excision for preoperative diagnosis of palpable lesions and those found by breast imaging, being a faster, less invasive and less expensive method than the surgical biopsy for diagnostic purpose [26]. A suspicious clinical-imaging diagnosis for breast cancer, but cytological and/or histological confirmed, can obviate the need for surgery in women with benign lesions and can optimize surgical procedures performed in women with breast cancer [11, 20, 26, 45, 55]. Selection between fine needle aspiration cytology and needle core biopsy is realized under clinical circumstances [55] and depends mostly on the pathologist abilities. [20, 45, 55]

Comparative with an excision biopsy needle aspiration cytology is characterized by simplicity, quickness and minimum discomfort of the patient with reduced costs [45, 55], having variable sensitivity and specificity; values of sensibility between 53-100% and specificity between 34-100% are quoted in the literature [18, 20, 46, 55]. Positive predictive value for needle aspiration cytology tends to be near 100% (99.6-100%) and the negative predictive value between 60-90% [23, 45, 56]. Needle core biopsy (NCB) requires local anesthesia and presumes image guidance in most situations [5, 26, 55], especially in non-palpable solid breast lesions. However it shows a higher diagnostic sensitivity (92-99%, with an average of 95%) [9, 19, 41], and a specificity and a positive predictive value about 100% [20]. This procedure tends to replace needle aspiration cytology and the excision biopsy in most institutions because it

---

### Table 1: Distribution of cases on mammographic and cyto-histological categories

<table>
<thead>
<tr>
<th>BI-RADS 1</th>
<th>BI-RADS 2</th>
<th>BI-RADS 3</th>
<th>BI-RADS 4</th>
<th>BI-RADS 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI-RADS 1</td>
<td>BI-RADS 2</td>
<td>BI-RADS 3</td>
<td>BI-RADS 4</td>
<td>BI-RADS 5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

---

### Table 2: Performance indicators obtained in our study

<table>
<thead>
<tr>
<th></th>
<th>Histological report (B category)</th>
<th>Cytological report (C category)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97.14</td>
<td>94.28</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>85.71</td>
<td>75</td>
</tr>
<tr>
<td>Accuracy</td>
<td>97.56</td>
<td>95.12</td>
</tr>
<tr>
<td>False negative rate</td>
<td>2.86</td>
<td>5.72</td>
</tr>
<tr>
<td>False positive rate</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
has a diagnostic accuracy almost identical to excision [47, 9, 11, 19, 32, 39, 41] and a definitive diagnosis is possible in 90-99% of cases [29, 46]. In our study both procedures have been done consecutively, the sampling of NCB specimens being followed by aspiration on the needle sheath used for biopsy. Although the entire cellular aspirates have been hemorrhagic microscopic examination of the smears (3-12 per case) permitted to establish a quickly cytological diagnosis, its performance indicators (sensitivity, negative predictive value, diagnostic accuracy) proving to be inferior to histological diagnosis obtained after the microscopic examination of the samples (Table 2).

Fine needle aspiration cytology is a very safe technique in experienced hands [3, 25, 51], but only occasionally allows a specific tissue diagnostic for a benign lesion [7, 11, 55] (Fig. 2) and in case of malignant lesions gives insufficient informations for a further therapeutic decisions, because it not allows a distinction between proliferative lesions with atypia, in situ lesions and invasive ones (Fig. 4), lesions which can be diagnosed on the base of cytological criteria combined with the architectural one [2, 7, 11, 24, 52, 55]. At the same time cytological atypia from the cytological specimens are not diagnostic, 45% of them being malignant lesions [7]. The diagnostic limits of fine needle aspiration cytology have determined Klein et al. [25] to suggest that this technique should be used supplementary and not complementary to histological examination and Pinder et al. [42] demonstrated that the preoperative diagnostic accuracy in breast lesions detected by screening may increase from 72% using only needle aspiration cytology to 90% if the method is used in combination with microbiopsy. Cohesive tissue samples are obtained by tissue microbiopsy (NCB) and allows a histological diagnosis using standard histological criteria [11, 20, 45, 55]: for these specimens is recommended a reporting system accessible to any pathologist no matter of his abilities and experience [16, 48]. Needle core biopsy requires a reduced degree of diagnostic expertise [46]: tissue fragments may be X-rayed when the lesion shows calcifications at the mammographic examination; simultaneously microscopic assessment of the cytological features and the architectural one allows a specific diagnosis of benignity (Fig. 2) and a quick recognition of a well differentiated tumor (lobular carcinoma, tubular carcinoma, cribriform carcinoma) (Fig. 3), but especially allows distinction between the proliferative high-risk lesions, in situ and invasive carcinomas [2, 5, 7, 11, 24, 46, 52, 55] (Fig. 4); in case of malignant lesions the type and tumor grading may be done with a reasonable accuracy degree [55] (Fig. 3 and 4), being possible the diagnostic of malignant lymphoma or breast metastasis [7], and immunohistochemical results about prognostic molecular factors shows an increased level of concordance with those obtained on the surgical excised samples [22, 55] (Fig. 5).

In our archive we included in C2 and C3 categories five cases and 1 case, respectively. In these situations the histological reports were described B2 and B3 categories and correlated with

![Fig. 5 - Immunohistochemical stains for hormonal receptors (same case: H.F., 55 years old): a. Cytology smears (ERx400, average receptor level by 10-15%); b. Histology sections (ERx100, average receptor level by 45-50%)](image-url)
clinical evolution and radiologic features (stationary lesions) at intervals of 6 months from the tissue sampling; on the base of cyto-architectural findings assessed on the tissue fragments we were sustained the diagnosis of fibroadenoma (2 cases), isolated breast fibrosis (2 cases), sclerosing lesion (1 case) and fibrocystic change (1 case). Only in 2 cases from 29 included in C5 category the histological findings of the biopsy could not certainly evaluate the tumor invasion (B5a+c categories); in the other 27 cases the microscopic examination of the NCB specimens have permitted a diagnosis of malignancy and assessment of tumor invasion (B5b categories - 24 cases and B5a+b categories - 3 cases), histological type and tumor grading. We consider that the hipocellularity of the cytology specimens and the lack of experience of the pathologist have determined the inclusion of 3 cases in C4 category, these one corresponding to malignant lesions on the biopsy (B5 category) (2 invasive carcinomas and 1 invasive carcinoma with associated in situ component), and 1 case in C3 category, this one proved to be on the biopsy a mixed invasive carcinoma: ductal and lobular (B5b category). We obtained unsatisfactory IHC results on cytology preparations because of hemorrhage and hipocellularity (Fig. 5). Also, because of different preparations, the immunohistochemical stains for hormonal receptors and other markers on cytology smears obtained by aspiration gave us results not comparable with those specimens obtained on biopsy, at this time having no schemes of quality assessment for reporting the hormonal receptors status on cytology smears [30, 46, 55].

The NCB technique followed by aspiration used in this study didn’t generate false positive results neither for cytological examination nor for histopathology. False positive diagnosis is extremely rare in case of NCB and can appear in case of sclerosing lesions [45] or in hystiocytic proliferations induced by radiotherapy [17]. In case of needle aspiration cytology the fibrocystic disease with marked epithelial proliferation, granulomatous mastitis, fibroadenoma and radiotherapy induced changes may cause false positive interpretations even for experienced cytopathologists [12, 45, 55].

Fine needle aspiration cytology is minimal invasive and well tolerated, but the false negative rate of the cytological reports is unacceptable increased (up to 31%) [1, 10]. This happens most times owing to the insufficient samples [5, 7, 26, 45], which frequently appears in small lesions [1], and is influenced by the nature of breast lesion: most of the malignant lesions mistakenly cytologically interpreted as benign lesions belongs to well differentiated tumors (tubular carcinoma, lobular invasive carcinoma, papillary carcinoma, cribriform carcinoma) [45, 46, 55] and less cellular tumors (carcinomas with extensive fibrosis) [38, 46]. In case of NCB most of false negative diagnosis, which appear with a frequency of 1-6% [10], is due to an insufficient sample from the lesion, situation frequently found in case of breast lesions with necrosis or extensive fibrosis or those with heterogeneous histological structure (in situ carcinoma, lobular invasive carcinoma, tubular carcinoma) [2, 20]. Technical accidents can’t be excluded, such as stereotatcic errors in case of mammographic guidance or sliding lesions owing to tissue elasticity [20, 45]. However, the drawing error decrease with the volume of the sample [20], number and size of fragments being correlated with the efficiency of the method [8, 28, 36]. One case from 41 included in this study, with benign cyto-histology after needle core biopsy followed by aspiration (C2/B3 category), but included in BIRADS 4 category (suspect for malignancy), proved to be a malignant lesion after the excision biopsy (invasive carcinoma). We consider this case of negative result a consequence of an insufficient sample from a small size breast lesion (10/7/5 mm) obtained by using a small caliber needle (18G) deviated because of tissue elasticity, the procedure being very difficult. The main cause which generated one of the false negative result for cytological examination (C3/ B5b category) seems to be the marked hipocellularity nature of the lesion (invasive carcinoma with abundant fibrotic stroma) (Fig. 3).

The false negative results can not be omitted and so is necessary to compare the results of needle aspiration cytology or needle core biopsy with clinical and radiological data applying the triple diagnosis strategy: after this will be decided either the surgical operation with or without primary medical therapy or the imaging follow-up of the lesion in a short (3 months) or a long range time (6 months - 1 year) [7, 21, 20, 26, 35, 45, 47, 54, 55]. The communication between the radiologist and pathologist is essentially for the recognition...
of adequate cito-histological specimens: the radiologist must describe the identified lesions and formulate a presumable diagnosis and the pathologist must mention if it is any concordance or discordance between microscopic findings and the imaging features of the lesion [7, 20, 21, 35, 47, 55]. If the imaging data and the histological ones obtained by NCB are concordant may be obtained comparable results with those of surgical biopsy [20]. Discordant data for percutaneous breast biopsy indicate the rebiopsy or surgical excision and have an incidence about 1-6%, being rarely for image-guided needle core biopsy than fine needle aspiration cytology [5, 26]. Lieberman et al. [27] have found a discordance rata about 3% in 1785 consecutive needle core biopsies. In other series [6, 13, 29, 33, 34, 40, 50] 11% cases from ultrasound-guided 14G needle core solid breast biopsies required rebiopsy: majority of the rebiopsies were done for atypical or high-risk lesions [5, 7, 26] and only in 0.5% of cases of Berg’s series [6] the lesion has been omitted. In our study discordances between clinical-imaging data and cyto-histological ones were noticed in 4 cases (9.75%). Owing to clinical circumstances (collateral breast neoplasm treated by chimiotherapy and radiotherapy) in 2 cases included in BIRADS 4/C2/B2 categories was recommended imaging follow-up and the lesions proved to be stationary at 3 months, 6 months and 1 year range. Excision biopsy performed in the other 2 cases of discordance between mammographic findings (BIRADS 4 and 3 categories) and those cyto-histological (C2/ B3 categories and C4/ B5, respectively) has confirmed the malignant nature of the breast lesions (invasive carcinomas).

Fine needle aspiration biopsy and needle core biopsy can be performed by surgeon, clinician or pathologist, but the efficiency of the cytological and histological result is increased if the procedure is done under image guidance (stereotactic, ultrasonographic) [5, 26, 45]. In Lorenzen series [31] NCB sensitivity for palpable breast lesions has increased from 79% for the biopsies guided by palpation to 98% for those performed under ultrasound-guidance. The global accuracy of the ultrason sound-guided biopsies is significantly better than stereotactic-guided [26, 37, 43, 44]. Among the institutions which have participated for RDGOV trial (Radiation Diagnosis Oncology Group V) the diagnosis accuracy for ultrasound-guided fine needle aspiration cytology was 77% with a false positive results ratio about 9%, while for NCB specimens was reported an accuracy about 97.5% without false positive results [13, 43]. The best results are obtained if multiple fragments are obtained under ultrasound guidance using 14G devices: Fishman et al. [14] has reported an accuracy about 96%, in other series [6, 13, 29, 33, 34, 40, 50] the sensibility was 95%, and Parker [40] has reported 100% concordance between the results of this procedure and the results of surgical excision.

**Conclusions**

Fine needle aspiration cytology and needle core biopsy represent usual methods of preoperative diagnosis in solid breast lesions [55] and successfully replace excision biopsy for diagnostic purpose. Cytological specimens’ interpretation obtained by fine needle aspiration cytology requires training and experience [11, 45, 55]. Needle core biopsy has gained in popularity over the last several years displacing fine needle aspiration cytology as the preferred diagnostic modality of palpable and non-palpable solid breast lesions prior to excision [4, 5, 7, 20, 26, 32, 45, 55]. Performing the biopsy under imaging guidance can ensure that the lesion was sampled.

The results of our study confirm that needle core biopsy (NCB) is characterized by diagnostic advantages and performance indicators better than fine needle aspiration cytology and we think that: assessment of NCB sections give more reproducible results and a higher accuracy of specific benign and malignant diagnosis obtained and, thus, a significantly lower rate of subsequent excision biopsies is required for diagnostic purposes; a core biopsy diagnosis of a specific benign lesion and distinction between true negative (specific benign lesion) and false negative (malignant lesions failed by drawing errors) results is an important advantage of this technique compared with fine needle aspiration cytology; needle core biopsy may distinguish between invasive and non-invasive cancers; NCB allows accurate immunohistochemical assessment of oestrogene and progesterone receptor status of all cancers diagnosticated, including women who elect not to have or cannot perform surgery.
Correlations between clinical, imaging and pathological data are obtained in multidisciplinary meetings in which the clinician, the surgeon, the radiologist and the pathologist establish a consensus about diagnosis and therapeutic strategy following defined protocols [55], the therapeutic options being able to be discussed with each patient [11, 20, 26, 45, 46, 55].

References

29

Needle core biopsy versus needle aspiration cytology in preoperative diagnosis of solid breast lesions


37. NHS Breast Screening Programme Guidelines for Cytology Procedures and Reporting in Breast Cancer Screening: Report by Cytology Sub-Group of the National Coordinating Committee for Breast Screening Pathology: NHSBSP Publication N. 22, sept 1993;


THE IMMUNOFLUORESCENCE IN THE ASSESSMENT
OF THE RENAL BIOPSY SPECIMENS

C. Ionescu¹, M. Hortopan², G. Ismail¹, E. Buzatu¹,
E. Buzuioc¹, V. Herlea², M. Voiculescu¹

¹Center of Internal Medicine - Nephrology,
²Department of Pathology, Fundeni Institute

Abstract  The renal biopsy is the principal method used in order to define the type of renal disease, especially when the preliminary clinical diagnosis is glomerulonephritis. The method provide sufficient accurate information that allows to establish a tissue diagnosis, to assess the severity and activity of the lesion ('grade') and to assess the amount of irreversible scarring ('stage'). The renal biopsy specimen must be examined by, at the very minimum, detailed light and immunofluorescence microscopic studies. Immunofluorescence microscopy is a routine part of the investigation of native renal biopsies. A basic panel of antibodies should be used for the detection of tissue deposits of IgG, IgA, IgM, and complement (usually C3).

Key words: immunofluorescence microscopy, renal biopsy, immune deposits, glomerule, mesangium, nephrotic syndrome

Address for correspondence:  Dr. Camelia Ionescu,  Center of Internal Medicine, 258 Fundeni Clinical Institute, Bucharest, Romania, Tel/Fax: 021-3180445, E-mail: camellia_aiлемac@yahoo.com
Immunohistochemical investigations of renal biopsy

This type of renal biopsy interpretation should be a routine part of the investigation of native renal biopsies. A basic panel of antibodies should be used for the detection of tissue deposits of IgG, IgA, IgM, and complement (usually C3). The most used antibodies include those directed against:
- C4 and/or C1q: to indicate classical pathway complement activation.
- C5b-9: to detect membrane attack complex deposition.
- Fibrin: to detect fibrinoid necrosis in vessels and glomeruli, and fibrin in crescents.
- Both Kappa and Lambda light chains: not only for myeloma kidney, but also the more subtle changes of light chain nephropathy.

Additional antibodies can be used at the nephrologist and pathologist’s discretion. In special case the list of antibodies that might be used include:
- Specific amyloid types.
- Specific chains of type 4 collagen (Alport's syndrome).
- Fibronectin (fibronectin glomerulopathy).
- Type 3 collagen (collagenous glomerulopathy).
- Viral antibodies.
- Tamm-Horsfall protein.

It is obviously difficult to detect the immunoglobulins in glomeruli due to the presence of abundant plasma proteins, which must be removed to avoid non-specific staining. This has resulted in a continuing popularity of frozen sections and immunofluorescence techniques in renal pathology, where they have been superseded in most other branches of immunohistochemistry by methods that are applicable to paraffin wax sections. (Tab. 1) In paraffin wax sections, the plasma proteins have been fixed in the tissues and must be removed by a digestion process rather than simple washing.

---

**Table 1**: Types of immunohistochemical investigations of renal biopsy

<table>
<thead>
<tr>
<th>Direct immunofluorescence</th>
<th>Immunohistochemistry using paraffin wax sections</th>
</tr>
</thead>
</table>

---

Immunofluorescence

This approach has the advantage of simplicity and reliability because plasma proteins can be removed from frozen sections simply by washing. The essential panel of antibodies against immunoglobulins and complement can be used with a direct immunofluorescence method, which is extremely simple and quick. Immunofluorescence has several disadvantages. A separate frozen specimen must be taken at the time of biopsy. Cryostat and epifluorescence microscopes are required. The preparations must be mounted in aqueous media and they are not permanent; exposure to light causes bleaching. This can be reduced but not eliminated by using specialist mounting media and storing the sections in the dark in a refrigerator. Ideally, relevant images should be stored, photographically or digitally.

**Immunofluorescence technique**

After it has been proper snap-frozen in liquid nitrogen, the tissue is then cut in a cryostat at a temperature between -25 and -20 C. The sections must not exceed 3-4 m in thickness. The slides are fixed for 5 to 10 minutes in acetone, dried at room temperature, washed in buffered saline, and covered for 30 minutes with a drop of fluorescein-labeled antiserum in a moist chamber that is light shielded. After three washes with buffer, the slides are mounted using buffered glycerol. The section is then examined using a fluorescence microscope equipped with appropriate excitation and barrier filters. The section should be photographed, because the fluorescence fades on exposure to both ultraviolet and incident light. The slides can be stored at 4 to 8 C for only 12 - 24 hours.

**Immunohistochemistry using paraffin wax sections**

A detection method applied to sections from the block that is re-used for routine microscopy has obvious advantages. It allows correlation with morphology and results in a permanent preparation that does not require special equipment or a separate sample for its production or
examination. However, immunofluorescence remains the method of choice in the majority of laboratories because of the technical difficulties of the alternative methods (3).

**Case report**

A 34-years-old female presented lower-extremity edemas. She had noticed the edemas over the past five years. She has never received any treatment in this period. Physical examination revealed an increase blood pressure of 150/90 mmHg and bilateral edemas at lower-extremity. The rest of his physical findings were unremarkable. Biological exam showed nephrotic syndrome (proteinuria: 5.7g/24h, hypoproteinemia: 5.9g/dl; hyposerinemia: 2.4g/dl, hypercholesterolemia: 260mg/dl), with normal renal function (serum creatinine: 0.8mg/dl, Cl creatinine: 100.1ml/min). Abdominal ultrasound showed normal kidneys.

A percutane renal biopsy was performed using a Bard pistol with a 16G needle. The renal biopsy specimen of 0.8cm length was then examined by immunofluorescence and light microscopy.

**Immunofluorescence:** diffuse deposits of IgA in the mesangium, forming discreet granules. IgG mesangial deposits are present in smaller amount. IgM, complement fractions and fibrinogen deposits are absent in this case. (Fig. 1).

**Light microscopy:** renal biopsy fragment with more than 20 glomeruli showed diffuse and global lesions. Most of the glomeruli present severe mesangial proliferation and free capillary lumen. Areas of sclerosis and hyalinosis involve segments of the glomeruli in 50% of them. An irregular and segmental glomerular basement membrane thickening occurs in some glomeruli. Also, some glomeruli present advanced lesions of sclerosis and presence of synechiae and cellular crescents. The immediately adjacent Bowman’s capsules are frequently thickened and multilaminated (Fig. 2, 3).

**Tubulo-interstitial lesions** are also present in this case. Scattered areas of interstitial edema and fibrosis, with interstitial inflammatory cell infiltrates predominate around the more severely damaged glomeruli.

In this case, the immunofluorescence pattern is...
typical for IgA nephropathy. The light microscopy showed a pattern of diffuse mesangial lesions with proliferation and sclerosis.

**Discussions**

IgA nephropathy can only be accurately diagnosed by immunohistochemical methods. The single constant findings in this disease is the presence of diffuse deposits of IgA in the mesangium, forming either discreet granules or large aggregates. IgG codistributes with IgA in more than one-half of the cases, but in smaller amounts. IgM has been reported in the mesangium in 50% of the cases. C3 is present in most of the patients with the same distribution as IgA. The presence of C1q and C4 is not common. Also, fibrinogen deposits are smaller and usually absent.

Although the disease was first described as a focal proliferative glomerulonephritis, it has been recognized that there are multiple histologic patterns of the disease. The World Health Organization working group on classification of glomerular disease has proposed the five categories of the IgA nephropathy (Tab. 2).

In the case presented immunofluorescence reveals the diagnosis of IgA nephropathy and light microscopy showed a histological pattern of diffuse mesangial lesions with proliferation and sclerosis (class IV - WHO classification).

**Conclusions**

The renal biopsy is the principal method used in order to define the type of renal disease, especially when the preliminary clinical diagnosis is glomerulonephritis. The renal biopsy provides the type of renal disease and accurate information in order to establish the therapeutic strategy and the prognosis(4-6).

In the case reported in this article the renal biopsy fragment was processed for light and immunofluorescence microscopy and reveal a histological pattern of class IV - WHO classification of IgA nephropathy. In IgA nephropathy the immunofluorescence is mandatory for the diagnosis and light microscopy provide the histological pattern of the disease.

**Bibliography**

5. Nair R, Walker PD. Is IgA nephropathy the commonest primary glomerulopathy among young adults in the USA? Kidney Int. 2006; 8
MORE TREATMENT CHOICES FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A CASE REPORT AND REVIEW OF THE LITERATURE

C.A. Dasanu¹, Elena Stoica-Mustafa², V. Herlea², D.T. Alexandrescu¹

¹Department of Hemato-Oncology, New York Medical College, NY, U.S.A.
²Department of Pathology, Fundeni Clinical Institute, Bucharest, Romania

Abstract

We present an illustrative case of a patient with advanced stage chronic lymphocytic leukemia (CLL), treated with both chemotherapeutic agents and monoclonal antibodies, in his first-, second-, and third-line therapies, with a reasonably good clinical response at each relapse. Further, we list some of the most commonly utilized modern therapies of this disease. Others are still in the process of development, giving even more hope to the CLL patients, their families, researchers, and clinicians in the nearest future. Entire concepts in CLL therapeutics have changed over the last a few years, therefore primary care providers need to be familiarized with the new agents, their mechanism of action, special handling and major toxicities.

Key words: Chronic lymphocytic leukemia (CLL), fludarabine, rituximab, alemtuzumab, cladribine, pentostatin

We certify that any affiliations with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, honoraria, expert testimony) is disclosed above. No financial support was used for this work.

Case presentation

A 64-year-old man presented to our clinic for the first time three years ago with the main complaint of profound weakness and exertional dyspnea. On physical examination, he was extremely pale and had a few small cervical nodes and a spleen that was palpable 3 fingerbreadths beneath the left costal margin. A complete blood count at that time revealed a hemoglobin level of 7.5 g/dl, a leukocyte count of 18,000/mcl, 90% of which were small lymphocytes with smudge cells present (nuclear remnants of cells destroyed while making blood smears), and a platelet count of 400,000/mcl. The peripheral smear is shown below (Fig. 1).

The peripheral lymphocytes were CD5+, CD19+, CD20 weakly positive and CD23+. Surface immunoglobulin type IgG was demonstrable with monoclonality for kappa light chains. The diagnosis of chronic lymphocytic leukemia...
(CLL), high risk by Modified Rai Staging System, was made. Patient was transfused with 3 Units of packed red blood cells and chemotherapy with IV fludarabine was started. Between August 2002 and March 2003, he received a total of 4 cycles of fludarabine with a good clinical response and near-resolution of his splenomegaly and anemia.

Patient remained asymptomatic until May 2004, when he presented with generalized lymphadenopathy and symptomatic splenomegaly. Further, 8 standard-dose weekly rituximab infusions were given and subsequently patient entered another partial remission. The second remission was, however, short-lived. In November 2004, he developed fever, night sweats and enlarged, painful left axillary lymphadenopathy. The lymph node biopsy showed diffuse small lymphocytic infiltrate. The peripheral lymphocytes were CD5+, CD20+, CD38+, therefore chemotherapy with fludarabine, cyclophosphamide and rituximab (FCR) was employed and continued for a total of 4 cycles. Although lymphadenopathy resolved, the B signs persisted.

In May 2005, patient was diagnosed with Pneumocystis carinii pneumonia (PCP) and treated with IV Bactrim and prednisone. Bactrim was continued orally as PCP prophylaxis. Five months after, he developed a right-sided pleural effusion. A pleural tap revealed small lymphocytes, consistent with his CLL. The bone marrow aspiration and biopsy showed a diffuse infiltrative pattern with small lymphocytes that were CD5+, CD19+, CD20, ZAP-70 positive. Further therapy with subcutaneous alemtuzumab is contemplated.

Newer therapeutic options and discussion

Cure of disease has been a fervent hope of many CLL patients, their families, researchers and clinicians. The increased longevity in general population is another factor that prompted the emergence of new, more effective chemotherapeutic options, along with other less toxic systemic treatments including monoclonal antibodies, or chemoimmunotherapeutic combinations. Entire concepts in CLL therapeutics have changed over the last a few years, therefore primary care providers need to be familiarized with the new agents, their mechanism of action, special handling and major toxicities. A few chemotherapeutic agents and two monoclonal antibodies are being introduced and more are on the horizon.

Fludarabine

This nucleoside analog, introduced earlier in Europe, has been increasingly used as first-line therapy in US. Fludarabine is delivered as an IV infusion daily for five days, every 28 days, repeated for 6-10 cycles, not to exceed one year of treatment because of cumulative myelotoxicity. There is potential for tumor lysis syndrome with its use and therefore good hydration, oral allopurinol, and close laboratory monitoring are warranted in the first two weeks of therapy. Fludarabine is the most active chemotherapeutic agent for treatment of CLL. It offers several advantages over the established alkylating agent, chlorambucil, which continues to be considered one of the first-line agents by many. (1) Thus, when tested against chlorambucil, fludarabine induced almost twice as many responses. Time to disease progression was also longer for fludarabine: 25 months versus 14 months. (2) Moreover, fludarabine provides effective salvage treatment for chlorambucil failures. Together with cyclophosphamide, it constitutes an efficient treatment for advanced CLL. (3) Patients with fludarabine-induced remissions of more than a year show reasonable response rates when retreated with fludarabine upon relapse. However, fludarabine also causes greater myelotoxicity and immunosuppression, resulting

Fig. 1 - Peripheral smear showing multiple small lymphocytes, smudge cells, and a variable degree of poikilocytosis, anisocytosis and hypochromia
in prolonged decreases in CD4 lymphocytes. Blood products should be irradiated to avoid transfusion-related graft-versus-host disease (GVHD). Just like in AIDS patients, prophylaxis against *Pneumocystis carinii* pneumonia is advisable until the CD4 lymphopenia resolves.

Coombs-positive hemolytic anemia is seen with an increased incidence after use of fludarabine, therefore its use is contraindicated in patients with prior history of autoimmune hemolysis. Reversible neurotoxicity is described, primarily in the form of somnolence and mild paresthesias.

**Rituximab**

This chimeric (mouse-human) monoclonal antibody has specificity against CD20, a surface antigen present on neoplastic and normal B-lymphocytes. It is thought to cause cell lysis via complement and antibody-dependent cellular cytotoxicity (ADCC) and to induce apoptosis by a direct mechanism. Response rates of 51% including 4% complete responses (CR) have been recorded while using Rituximab as a single drug in the first-line setting. (4) Partial responses (PR) have been reported in 25% of the CLL patients previously treated with fludarabine. (5)

Rituximab is used in standard dosage of 375 mg/m² IV weekly or in escalating dosing. The latter might produce higher responses, yet expense is significant. An impressive response rate of 90% including CR in 43% was achieved by the concurrent use of fludarabine and rituximab. (6) Furthermore, rituximab has also been utilized in combination with fludarabine and cyclophosphamide for both initial and salvage therapy of CLL, with response rates of 90% (60% CRs), but the effect of such combination on survival is uncertain. (7)

Moderate to severe infusion reactions are seen in approximately 90% of patients and therefore pretreatment with acetaminophen and diphenhydramine is recommended. Once such reaction have occurred, interrupting the infusion, administration of IV steroids, and restarting the drug at half the previous rate have all been shown to be effective. Myelotoxicity is minimal with rituximab. Severe cytopenias are seen on rare occasions with its use and are presumed to have an immunological basis.

**Alemtuzumab**

It represents another chimeric (humanized) monoclonal antibody targeting CD52, a surface glycoprotein expressed on normal and neoplastic B and T lymphocytes. Alemtuzumab is indicated for use in patients with fludarabine-refractory CLL and a response rate of 33% has been reported in that setting. (8) A recent study has shown that eradication of minimal residual disease in B-CLL is possible after alemtuzumab therapy and it is associated with prolonged survival. However, not only that alemtuzumab is myelosuppressive, but it also induces profound and prolonged immunosuppression. Opportunistic infections are common, including cytomegalovirus and fungi. 1 tablet DS of trimetoprim/sulfamethoxazole daily 3 days/week and famcyclovir 250 mg BID should be started with the initiation of the drug and continued until CD4 count is at least 200/mcl. All blood products should be irradiated before use. Severe anemia, neutropenia, and thrombocytopenia lasting 3 to 4 weeks are commonly reported; fatal pancytopenia is rare.

Infusion reactions should be handled in the manner described with rituximab. Fewer reactions are seen with the subcutaneous administration. The cost of the drug is significant.

**Future options**

Drugs on the horizon include other nucleoside analogs, two of which - cladribine and pentostatin - are in phase 3 clinical trials. Both drugs are considered "standard of care" in hairy-cell leukemia. They are being extensively studied in CLL in many European and US centers. Results of early trials appear to show their efficacy to be comparable and their toxicity profile to be similar to that of fludarabine. (10) Lumiliximab, an anti-CD23 monoclonal antibody, is currently in early-phase testing. Various combinations of alemtuzumab and/or rituximab with conventional chemotherapy agents as well as with each other are also being investigated. (11)

**References**


PRIMITIVE Rhabdomyosarcoma
of the Diaphragm, Embryonal Type

Raluca Cristea¹, V. Herlea¹, Monica Hortopan¹, Mihaela Mihai¹³,
C. Pechianu¹, Doina Hrehoret², Carmen Ardeleanu³,
Camelia Dobrea¹, I. Popescu²

¹Pathology Department of Fundeni Hospital, Bucharest
²General Surgery and Liver Transplantation Centre of Fundeni Hospital, Bucharest
³National Institute "Victor Babes", Bucharest

Abstract Rhabdomyosarcoma is a childhood tumor. (RMS) is the most common diagnosed sarcoma in children and adolescents, and frequently in young adults, with having bimodal age distribution: the first peak of incidence at children between 2 and 5 years old, the second peak at adolescents between 15 and 19 years old. RMS is diagnosed in 50% of cases to patients less than 5 years old, and about 6% in their first year of life. In adults, it is seen after the age of 50 and is more frequent in males (1). The age of RMSs diagnosis tend to correlate to the site of primary disease and tumor histology. RMS may occur at any site, but primary tumors are more commonly located in head and neck (42%), urogenital tract (34%), and extremities (11%). Morphologically, there are 3 histologic subtypes: embryonal (the most frequent - about 66%), alveolar, and pleomorphic (2; 24). We present the case of a 4-years old boy, hospitalized General Surgery and Liver Transplantation Centre of Fundeni Hospital, for a voluminous tumoral mass attached to the left diaphragm, marking the surface on the left lobe of liver. Histological exams of the specimen extracted on laparoscopy was realized in the Pathology Department of Fundeni Hospital and revealed: an undifferentiated malignant tumor, with small cells; correlated with immunohistochemical test results the diagnosis established is embryonal RMS. It is delivered a chemotherapy for 7 month. The follow-up of patient during the therapy revealed reduction of tumor dimensions, so it is decided surgery and tumor resection. A macroscopic and microscopic exam reveals malignant mesenchimal with small cells diffusely involving the diaphragm, without invading hepatic parenchyma. Conclusion: primitive diaphragmatic RMS, embryonal type. (Conclusions: 1). The specificity of the case consist in the tumor location; primitive diaphragmatic RMS is a very unusual entity. 2). The pathological exam establish an accurate diagnosis, excluding other diseases as are Ewing sarcoma, leukaemia, neuroblastoma, non-Hodgkin lymphoma, peripheral primitive neuro-ectodermal tumors (PNET) and lead to a correct therapy.

Key words: primitive rhabdomyosarcoma, embryonal type, diaphragm

Address for correspondence: Dr. Vlad Herlea, Department of Pathology, Fundeni Clinical Institute, Fundeni street 258, 022328, Bucharest, Romania; E-mail: herlea2002@yahoo.com
Introduction

RMS is the most common mesenchymal tumor at children and young adults. RMS is a malignant tumor that develops from primitive mesenchymal cells. The tumor frequently develops in sites that muscular tissue is not usually present. (2)

Rhabdomyosarcoma may occur at any site, but primary tumors are more commonly located in head and neck (42%), urogenital tract (34%), and extremities (11%). The presence of striated muscle tissue is not a prerequisite for its development (1, 7).

Alveolar tumors tend to occur primarily in trunk and extremities, whereas embryonal tumors are more often found in the head and neck or genitourinary and paratesticular sites. The pleomorphic type is specific for adults and is placed specially in extremities. The embryonal type develops from the undifferentiated mesoderm and usually is located in the regions of the head, neck, retroperitoneum, biliary ducts and urogenital tract. Rarely is located to extremities. The botryoid subtype develops in the submucosa of cavitory organs (vagina, bladder, biliary ducts) and has a gross typically aspect of "grapes", and microscopically tumoral cells with evident myogenesis in their cytoplasm. The spindle cell subtype is usually located paratesticular, and microscopical features reveal scanty rhabdomyoblasts (2, 24).

Primary tumors of the diaphragm are uncommon entities, and diaphragmatic rhabdomyosarcoma is an extremely rare tumor. This primitive site promotes adjacent structures invasion, as are: lungs, great vessels, and/or liver. In diaphragmatic location the symptoms are: abdominal pain, vomiting, constipation, pneumothorax, hemothorax. Primary tumors of the diaphragm are uncommon entities, and diaphragmatic rhabdomyosarcoma is an extremely rare tumor (Table 1).

The clinical features of RMS vary with age of the patient, site of the tumoral mass, and presence or absence of metastatic disease. Gross features of the tumor are not characteristic. They are generally firm, occasionally nodular, and variable in size. There is a tendency to form pseudocapsules around the tumor. The tumor can be so large that determination of the site of origin may be not possible initially. The majority of the symptoms are related to the tumor compression on adjacent organs, but sometimes RMS is occasionally diagnosed in an asymptomatic patient. Diagnosis is usually made by the discovery of a visible or palpable mass or by interruption of normal function of the adjacent organs.

Rhabdomyosarcoma is a pediatric disease specified by the recurrent chromosome translocations t(2,13)(g35,14) and rarely t(1, 13)(g36, g14). These translocations result in the formation of the PAX3-FKHR and PAX7-FKHR fusion genes, which are thought to play a causal role in the genesis of this disease (13).

Case report

We present a 4-year old boy, S.V., hospitalized in 2004, October, for following accuses: abdominal pain, vomiting, subfebrility. Clinical examination reveals: a feverless patient, good general state, generalized microadenopathy, tumoral mass in epigastic and right hypogastric region which, on
palpation has a irregular surface, poorly delimited; there are no other modifying on clinical examination. Complex clinical and paraclinical exams were done. Laboratory exams showed high levels of: erythrocyte sedimentation rate ESR (44/1h; 83/2h), fibrinogen (529mg/dl), LDH (333U/l), C reactive protein (43,3mg/dl); anemia (RBC =10,5g/dl). Chest X-ray reveals no changes. Computerized tomography scan (CT) detects a tumoral mass on the left lobe of the liver, having dimensions about 10/11 cm.

The patient is hospitalized at General Surgery and Liver Transplantation Centre of Fundeni Hospital, where on October 18, 2004, was proceeded an exploratory laparotomy which reveals: tumoral mass on the left lobe of the liver, invading gastric body and diaphragm; peritoneal carcinomatosis nodules which were extracted for biopsy; it was effectuated an intraoperative upper digestive endoscopy which shows normal aspect.

Histological exams of the specimen extracted on laparotomy was realized in the Pathology Department of Fundeni Hospital and revealed:
- Macroscopically: 2 nodular masses with 0,8 cm and 1 cm diameters;
- Microscopically: formed by small, round cells and spindle-shaped cells, embryonal type, disposed into a lax stroma. (Fig. 1, 2)

Conclusion: metastatic mesenchymal hepatoblastoma.

For a certain diagnosis there are recommended immunohistochecmical tests, which were effectuated to 'Victor Babes' National Institute, and revealed:
- VIM(+) (Fig. 3); ACT(+) into the vessels, (-) into the tumor; EMA(+) in the periphery resting epithelial structures and (-) into the tumor (Fig. 4); CK8(-); S100(-); AFP(-) (Fig. 5); KL1 (-); CEA(-); OCH1E5(-) (Fig. 6); beta-HCG inconclusive;
- other tests: desmina diffusely (+) (Fig.7); VIII factor (-); CD7(-); CD34 (+) into the vessels, CD31 (+) into the vessels, poorly (+) into the isolated tumoral cells;
- Myogenina frequently (+) into the tumoral cells (Fig. 8).

Conclusions: immunohistochemical tests are compatible with a metastases from a rhabdomyosarcoma, embryonal type.

October 2004 -March 2005 the patient comes every month for chemotherapy and evaluation: laboratory exams, chest X-ray and abdominal ultrasonography. It is delivered chemotherapy, by IVA-SFOP'95 therapy protocol.

The patient followed for 7 months this multidrug therapy, and, as a result, by paracical evaluation it is observed a significant reduction in tumor dimensions, from 10/11cm to 5/6cm.

It is to notice that during this time interval there were no other pathological adjustments on the chest X-ray. Laboratory exams show a significant improvement of biological parameters (RBC, fibrinogen, ESR), thus, on April 18, 2005, the patient suffers a new surgical intervention. Intraoperative it is observed (Fig. 9):
- tumoral mass attached to the left diaphragm, with extrinsic growth, marking the surface on the left lobe of liver, without invading the liver;
- an epiploic nodule.

Histological exams of the specimen extracted on laparotomy was realized in the Pathology Department of Fundeni Hospital and revealed:
- Macroscopically: fragment of diaphragm adhering to the left lobe of the liver (90g) by a solid-cystic proliferation, dimensions = 9/6/5cm, having a gelatinous content, alternating with tan-yellow solid areas (Fig. 10);
- Microscopically: diffuse malignant mesenchymal proliferation with small, round-ovoid cells, with foamy cytoplasm, small, tachricromatic nuclei, with 5-7 mitoses/10 HPF, disposed into a lax, mixoid stroma (Fig.11, 12).

Conclusion: Diaphragm rhabdomyosarcoma, embryonal type, the diaphragm being the primitive site of the tumor.

 Discussions

RMSs belong to the class of "small round blue cell tumors" of childhood. Differential diagnosis includes: Ewing sarcoma, leukemia, neuroblastoma, non- Hodgkin lymphoma, and peripheral primitive neuroectodermal tumors (PNET). RMS is a primitive malignant soft tissue sarcoma that recapitulates the phenotypic and biologic features of skeletal muscle and has 3 principal subtypes: alveolar, embryonal (with botryoid and spindle cell subtypes)and pleomorphic (2).
Primitive rhabdomyosarcoma of the diaphragm, embryonal type

Fig. 1 - HE stain x 400

Fig. 2 - HE stain x 200

Fig. 3 - Vimentine (+) x 200

Fig. 4 - EMA x 100, (−) in tumor, (+) in mesothelium

Fig. 5 - AFP (−) x 100

Fig. 6 - OCH1E5(−) x 100
Fig. 7 - Desmine(+) x 200

Fig. 8 - Myogenine(+) x 200

Fig. 9 - Tumoral mass - surgical specimen intraoperative view

Fig. 10 - Tumoral mass - gross view - transversal section

Fig. 11 - Tumor of diaphragm (HEx400)

Fig. 12 - Tumor of diaphragm (HE x100)
The embryonal subtype is most frequently observed in children. Embryonal tumors are characterized by variable amounts of small, round or spindled-shaped cells, with variable cellularity. Individual rhabdomyoblasts are eosinophilic, and cross-striations are seen in 50% to 60% of tumors (25, 26).

A sarcoma should always be considered in the differential diagnosis of any soft tissue mass in children (2, 3). All patients with a RMS should have a complete blood count, liver function tests, a computerized tomography (CT) scan of chest, pelvis and abdomen, a bone marrow aspiration and biopsy.

RMS require a multidisciplinary approach involving surgeons, oncologists, and pathologists. Prior to institute the therapy, an extensive evaluation should be done, to determinate the extent of the disease, and that should include: chest X-ray; computed tomography (CT) scan of chest, abdomen, an pelvis with intravenous, oral, ar rectal contrast; bone marrow aspiration and biopsy; magnetic resonance imaging (MRI).

All children with RMS require multimodality therapy. This involves: surgical resection, if possible, followed by chemotherapy, followed by second-look surgery for some patients with initially unresected tumors, and radiation therapy. All children with RMS should receive chemotherapy with the quantity an duration dependent on a risk factors analysis (4, 27). Chemotherapy is the primary treatment for patients with metastatic disease at presentation. The common used drugs are a combination (multidrug therapy) of: Cyclophosphamide, Vincristine, Actinomycine and Doxorubicin, Cisplatin, Adriamicyn and Isofosfamid. A recent analysis has proved that children with embryonal disease, with complete resection but with groo residual disease, younger than 10 years of age, have a substantially improved outcome after multidrug chemotherapy (6-11).

Embryonal RMS, botryoid and spindle cell subtypes, have a highly favorable prognosis, and embryonal RMS not otherwise specified has an intermediate prognosis. The prognosis for children and adolescents with RMS is related to the site of origin, respectability, presence of metastasese, number of metastases sites, and histopathology (6-11).

RMS occasionally arise in sites other than those mentioned above. Such a very unusual site of RMS is the diaphragm. Patients with these tumors often have locally advanced disease that is is not grossly respectable initially due to fixation to adjacent vital structures suchas the lung, great vessels, and/or liver. In the circumstance, chemotherapy should be initiated after diagnostic biopsy, with the intent to try remove residual tumor at a later date (4, 5).

The incidenceThe authors report a case of primary rhabdomyosarcoma of the diaphragm, an extremely rare presentation with only 14 cases reported in the literature (Table 1). Primary rhabdomyosarcoma of the diaphragm presenting as an epigastric mass is extremely rare, with only three published cases. We highlight the unique imaging features in a 2(1)/(2)-year-old boy which predicted the correct anatomical site preoperatively. Understanding of this rare tumour and its imaging characteristics should help in differentiating it from other more common tumours in this location, especially primary hepatic tumours (12).

There was reported some other cases of primary RMS of the diaphragm (14-23):
- 2004 - pleomorphic RMS
- 2003 - 4 years old male- unclassified RMS
- 2002 - 4-years old female- alveolar RMS
- 2000 - 18 years old male- unclassified RMS
- 1999 - 3 years old male- embryonal RMS
- 1998 - 2 years old male- unclassified RMS
- 1993 - 20 years old female- pleomorphic RMS
- 1988 - adult, male- embryonal RMS.

Conclusions

1. The specificity of the case consist in the tumor location; primitive diaphragmatic RMS is a very unusual entity.
2. The pathological exam establish an accurate diagnosis, excluding other diseases as are Ewing sarcoma, leukaemia, neuroblastoma, non-Hodgkin lymphoma, peripheral primitive neuroectodermal tumors (PNET) and lead to a correct therapy.
References


29) Sternberg: Surgical Pathology - 1996???


Instructions for Authors

The Annals of Fundeni Hospital will consider for publication articles of clinical research in all medical fields. Manuscript categories are: Original Articles, Reviews, Case Reports, Special Articles, Medical Personality, Leading Article. The Review’s mission is to keep the practitioner informed of the advancements in all medical fields.

Papers that have already been published or are under consideration for publication in other journals will not be accepted. Authors must send a signed ‘Authors’ Responsibility Form to the Journal’s editorial office separately from the manuscript. Statements and opinions are the responsibility of the authors.

All manuscripts may be submitted via e-mail or at the editorial office, on electronic media. All correspondence thereafter is carried out by e-mail.

Before submission, prepare the manuscript completely with a word processor (any standard word processing software may be used). Arrange the manuscript as follows: title page, abstract and key words, introduction, methods, results, discussion, acknowledgements, references, figure legends, tables and figures.

Peer-review

Submitted manuscripts are first read by the editors. Some papers may be declined at this stage. The others will be sent for peer-review to two external referees usually selected from among the specialists in the Editorial Board of the Journal. The editors decide whether to accept or reject based on the referees’ recommendations. Submissions will be assessed primarily on their scientific validity and merit, not on their grammatical quality. Originality and clinical impact are essential for acceptance, and the descriptions of the following points are critically evaluated in original articles: the study rationale, trial design, and number of cases, approval of local ethical committees and informed consent by patients, precise data presentation and justifiable conclusions, selection criteria of cases, efforts to eliminate possible biases in retrospective analysis, justifiable conclusions, clinical impact of the study, statistical evaluation of the study.

Preparation of the manuscript

Use a large, clear font (e.g. 12-point or larger Times New Roman or Arial) and double-line spacing throughout.

The title page should carry a) the title of the article; b) authors’ names with institutional affiliations; c) corresponding author’s name with phone and fax numbers, street address and E-mail address; e) a running head of no more than 40 characters including spaces; f) an abstract of no more than 250 words. Do not use abbreviations or references in the abstract. The abstract of an original article should be structured into four paragraphs with headings of Background, Methods, Results and Conclusions.

Provide three to six key words for indexing purposes. Use terms from the medical subject headings (MeSH) list of Index Medicus.

Provide information concerning grants, contracts and other forms of financial support and the name(s) of institution(s) at which the work originated.

Under a heading of ‘address for correspondence’, provide the full name, exact mailing address with postal code, telephone and fax numbers, and e-mail address of the author to whom communication, proofs and requests for reprints should be sent.

Acknowledgements

Individuals who contributed significantly to the research or preparation of the manuscript may be acknowledged in this section.

References

Identify references in the text by Arabic numerals in parentheses on the line. References are typed, double-spaced, separate from the text. Number references consecutively in the order in which they are first mentioned in the text. The style and punctuation of references should be according to the style used in Index Medicus, National Library of Medicine.

Tables

Number tables consecutively in the order of their first citation in the text and supply a brief title for each. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all nonstandard abbreviations that are used in each table.

Figures

Submit three sets of unmounted glossy proofs and drawings. Artwork will not be returned.

Figures should be professionally drawn or computer-generated; freehand or typewritten lettering is
unacceptable. Letters, numbers and symbols should be clear and even throughout and large enough that when reduced for publication each item will still be legible. The first author’s last name, the figure number and the location of the ‘top’ must be indicated on the back of each figure. Symbols, arrows, or letters used in figures should contrast with the background. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend. Enough information should be given to allow interpretation of the figure without reference to the text. Written permission must be obtained from the publisher to reproduce any previously published figures. Cite the source of the figure in the legend.

Color photographs will be published free of charge.

**Statistics**

Describe which statistical methods were used for which analyses.

**Reprints**

Authors are provided with 10 reprints of each published paper free of charge.

Additional reprints are available at the author’s expense.

**Editorial Office**

Papers submitted for publication should be addressed to:

**Editor: Irinel Popescu**

Department of General Surgery and Liver Transplantation, Fundeni Clinical Institute, 3rd floor, 258 Fundeni Rd., Bucharest, Romania

Tel./Fax: +4021-318.04.17.

E-mail: irinel.popescu@icfundeni.ro