Pharmacogenomics

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Summary

Inherited variation of single genes coding for protein involved in drug matabolism, drug transport and drug target were found since long time to influence the response to drug administration. However, for many years, Pharmacogenetics has studied single gene affecting different aspect of drug disposal or effect. Nowadays, however, Pharmacogenomics is analysing the polygenic inheritance of drug response by the genomic wide-search of single nucleotide polymorphisms associated with different aspects of drug action. This approach in long term may led to the development of personalized medicine, identification of therapies that target specific subpopulation, improvement in the identification of the mechanism of drugs action, thereby allowing also to the development of new drugs.

Introduction

The recent progress in genomics has determined a strong drive to Pharmacogenomic development. Most investigators use the expressions pharmacogenetics and pharmacogenomics interchangeably. Others, however, considers pharmacogenetics the science which studies the inherited variation of a single gene implicated in a pharmacological response, whereas pharmacogenomics deals with many genes or the entire genome [1].

The effect of medications are influenced by many factors including age, organ function, concomitant therapy leading to drug interactions and the nature of the disease. However, recent studies have demonstrated that the interindividual differences in drug response are mainly related to sequence variations in genes encoding drug metabolising enzymes, drug transporter or drug targets.

Polymorphism in genes encoding drug-metabolising enzymes: Phase I

The enzymes involved in drug metabolism are divided into two categories according to their involvement in phase I reaction (oxidation, reduction and hydrolysis) or phase II conjugation reaction (e.g. acetylation, glucuronidation, sulfatation and methylation). The name of these pathways are purely historical, so that phase II can precede phase I and occasionally occur without phase I.

One of the first examples of pharmacogenetics, which was described approx 40 years ago, regards the hydrolysis of the muscle relaxant succinylcholine by butyrylcholinesterase (pseudocholinesterase). Approx 1:3500 normal white subjects are homozygotes for a gene variant resulting from an amino acid substitution (e.g. Aspargine to Glycine at pos 70) which codes for an enzyme unable to hydrolyze succinylcholine, thereby prolonging the drug-induced muscle paralysis and consequent apnea. Screening for the presence of this variant can be easily carried out by evaluating the percentage of inhibition of the enzyme by the local anesthetic dibucaine (the percentage inhibition is indicated as the dibucaine number) which is lower for the variant as compared to the wild-type enzyme (reviewed in Kalow and Grant [2]).

The cytochrome P450 enzymes, a superfamily of microsomal drug-metabolising enzymes, are the most important of the enzymes catalysing phase I drug metabolism. One member belonging to this family namely the cytochrome P450 2D6 (CYP2D6) is one of the best know example of genetic variation in drug-metabolising enzymes (*Table 1*). The CYP2D6 genetic polymorphism was discovered by noting marked differences in the pharmacokinetic and therapeutic effects of drugs metabolyzed by this enzyme. Approx 7% of subjects of Caucasian origin were found to have a deficiency of the capacity to oxidize the antihypertensive drug debrisoquine. The rates of debrisoquine to its metabolite 4-hydroxy debrisoquine in urine indicates the extent of oxidation of debrisoquine. Subjects with poor metabolism have therefore high ratio and are thus exposed to overdosing and adverse effects (reviewed by Weinshilboum [3]; Meyer [4]; Tribut et al [5]). Subsequently the gene encoding CYP2D6 has been cloned and the polymorphic variants responsible for its low activity have been molecularly defined. More than 75 CYP2D6 alleles have been described. The most frequent mutation, responsible for low activity is an adenosine replacement of guanine at position 1934, at the limit between intron 3 and exon 4, which produces a splicing defect leading to a truncating and inactive protein containing 181 AA instead of the 457 of the wild type. Ultrarapid metaboliser have, on the contrary, multiple copies (0-13) of CYP2D6. Variations of the number of copies of CYP2D6 have a striking effect on the pharmacokinetics of the antidepressant drug nortriptyline. A microchip for determining the polymorphism of CYP2D6 relevant to the clinic has been recently set up. Subsequently other cytochrome P450 isoforms including 2C9, 2C19, and 3A5, which metabolize a very large number of drugs have been characterised (reviewed by Weinshilboum [3]; Meyer [4]; Tribut et al [5]).

Polymorphisms in genes encoding drug-metabolizing enzymes: phase II

One of the early examples of inherited variation in phase II drug metabolising enzyme is represented by the N-acetylation of isoniazide (*Table 1*).

Acetylation of isoniazide shows a bimodal distribution (fast and slow acetylator), which results from polymorphism of the N-acetyltransferase 2 gene (NAT1). Large ethnic difference exist in the frequency of the rapid and slow acetylator alleles (many variants).

One of the most developed example of clinical pharmacogenetics (reviewed by Weinshilboum [3]) regards the genetic polymorphisms of thiopurine methyltransferase (TPMT). TMPT catalyzes the 5-methylation of the thiopurine drugs, azathioprine, mercaptopurine and thioguanine, which are used in the treatment of childhood leukemia and a number of autoimmune disorders. The thiopurines require metabolism to thioguanines (TGN) which exert their activity through incorporation into DNA. These agents are inactivated mainly via methylation by TMPT. TMPT

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TABLE 1 - *Pharmacogenetics of drug metabolism.*

Drug-Metabolizing Enzyme	Frequency of variant poor-metabolism phenotype %	Representative drugs metabolized	Effect of polymorphism
PHASE I			
Cytochrome P-450 2D6 (CYP2D6)	7	Holoperidol	Enhanced drug effect
		Serotonin reuptake inhibitors	Enhanced drug effect
		Antiarrhythmic drugs	Enhanced drug effect
		Nortriptyline	Enhanced drug effect
		Codeine	Decreased drug effect
Cytochrome P-450 2C9 (CYP2C9)	3	Warfarin	
		Phenytoin	
		Tabultamide	
		Losarton	Enhanced drug effect
Cytochrome P-450 2C19 (CYP2C19)	3	Omeprazole	Enhanced drug effect
		Diazepan	
Dihydropyrimidine dehydrogenase	1	Fluorouracil	Enhanced drug effect
PHASE II			
N-Acetyltransferase 2	52	Isoniazid	Enhanced drug effect
		Hydralazine	
		Procainamide	
Uridine diphosphate-glucuronosyltransferase 1A1 (TATA-box polymorphism)	10.9	Irinotecan	Enhanced drug effect
		Bilirubin	Gilbert's syndrome
Thiopurine S-methyltransferase	Approximately 1 in 300 whites	Mercaptopurine	Enhanced drug effect
		Azathioprine	(toxicity)
Catechol O-methyltransferase	25	Levodopa	Enhanced drug effect

activity is highly variable: 90% of individuals have high activity, 0.3% have low activity and 10% have intermediate activity. TMPT activity is inherited as an autosomal codominant trait. Subjects inheriting TMPT deficiency accumulate excessive cellular concentration of TGN which led to myelosuppression. The molecular basis of these phenotype have been elucidated. Three alleles 2, 3A and 3C account for 95% of intermediate or low activity. Two types of TMPT analysis are at present time used in oncology, one based on the determination of TMPT red cell enzymatic activity and one based on allelic-specific PCR, which identifies the most common mutations. These tests allow to personalize the dose of thiopurine drugs, thereby avoiding toxicity as well as reduced efficiency (reviewed by Weinshilboum [3]; Meyer [4]; Tribut et al [5]).

Genetic polymorphisms of drug transporters

The best example of genetic polymorphisms influencing drug transporters regards the P-glycoprotein, a member of the ATP binding cassette family, which is encoded by the ABCB1 gene (also called MDR1). The principal function of MDR-1 is the energy-dependent cellular efflux of many substances (bilirubin, many anticancer drugs, cardiac glycosides, immunosuppressive agents, glucocorticoids, HIV-protease inhibitors). MDR1 has a role in the excretion of xenobiotics and metabolites into urine, bile and the intestinal lumen. A synonymous single nucleotide polymorphism in exon 26 (3435C →T) is associated with variable expression of MDR1 in the duodenum. Patients with the TT genotype have half the expression than those with the CC genotype. In TT subjects digoxin have higher bioavailability (reviewed in Meyer [4]; Tribut et al [5]; Evans and McLeod [6]).

Genetic polymorphisms of drug targets

Genetic polymorphisms in receptors (drug targets) have a marked effect on drug response. Among the best examples are sequence variants in the gene for the β_2-adrenoreceptor, affecting the response to β_2 agonists, by altering the process of signal transduction mediated by these receptors. Single Nucleotide Polymorphism (SNP) resulting in an Arg to Gly at codon 16 and/or in Gln to Glu at codon 27 are relatively common and are associated with altered expression, down-regulation or coupling at the receptor in response to β_2 agonists. For example, FEV1 after a single dose of albuterol was higher in subjects with the Arg/Arg phenotype at codon 16 as compared to those with the Gly/Gly phenotype. However, those with the Arg/Arg phenotype showed marked desensitization, thereby being at risk for deleterious or non-beneficial effects from regular therapy with inhaled β_2 agonist (reviewed in Meyer [4]; Tribut et al [5]; Evans and McLeod [6]).

Another pharmacogenetics example, very useful to know in clinical practice, is related to malignant hyperthermia (MH). MH, usually a dominant trait, occurs in 1:15.000 children and is characterized clinically by muscle rigidity, elevation of body temperature, acidosis, tachycardia following the administration of several substances used in anesthesia, including halothane and succinylcholine, which led to a pathologic elevation of

ionized calcium in the sarcoplasma. Most commonly the molecular defect resides in a mutation (C1843T), which gives rise to Cys for Arg substitution at position 614 in the ryanodine receptor, the calcium release channel of the sarcoplasmic reticulum.

Genetic polymorphisms in disease-modifying genes or treatment-modifying genes influencing the drug response

Several polymorphisms with an indirect effects on drug response regards proteins not affecting the metabolism or transport of a drug as well as not being a component of the drug target. One well know example concerns the inherited polymorphisms in coagulation factors (prothrombin, factor V Leyden) which predisposes women taking oral contraceptives to the development of deep-vein thrombosis.

A second example, of great relevance in practice, is related to genetic polymorphisms in cellular ion transporter, which predisposes patients to adverse effect following specific drug administration. For instance variation in KCNE2, a gene coding for a membrane subunit which assembles with Herg to form I_{k2} potassium channel, predispose patients with the long QT syndrome to develop fatal cardiac arrhythmia following clarithromycin administration (reviewed by Evans and McLeod [6]).

Polygenic determinants of drug response

We have so far examined the effect of single polymorphisms of a gene on drug response. However, most likely each individual carry many polymorphisms in different genes coding for different protein involved in the response to a drug. Therefore the response to a drug appear to be polygenic and can be considered a complex trait (reviewed by Evans and McLeod [6]).

Individualized medicine with pharmacogenomics

The identification of polygenic determinants of drug response can be obtained by genomic-wide search of SNP associated with drug effects. An alternative methodology is the candidate gene approach, which is based on the analysis of association of drug effect/toxicity with genes previously found to affect the disposal or the response to a drug. Both methodologies have limitation and advantages. These studies can be carried out by obtaining genomic DNA from patients enrolled in phase III trial of a newly developed agent and search for genetic polymorphisms associated with toxicity or variation in the drug response. Genomics can also be used to identify new drug target. For instance a genomic approach trough the study of gene expression can discover genes that are under or overexpressed in cancer cells that are sensitive to a anticancer agent compared with those that are resistant. The product of such overexpressed genes represent targets for development of future inhibitors that could have the potentiality to reverse the drug resistant phenotype (reviewed by McLeod and Evans [7]; Goldstein et al [8]; Evans and Relling [9]). These approaches can led to the development a personalized medicine, identification of therapies that target specific subpopulation and discovery of new drugs. Before the application of pharmacogenomics to clinical practice careful attention to ethical aspects should be considered to avoid abuse of genetic information against individuals.

References

[1] Nebert DW, Jorge-Nebert LF. Pharmacogenetics and pharmacogenomics. In: Emery and Rimoin's, Principles and Practice of Medical Genetics, New York: Churchill Livingstone, 2002, 590.

[2] Kalow W, Grant DM. Pharmacogenetics. In Scriver CR, Beaudet AL, Valle D, et al. The Metabolic & Molecular Bases of Inherited Disease, New York: McGraw-Hill, 2001, 225.

[3] Weinshilboum R. Inheritance and drug response. N Engl J Med, 2003, 348:529.

[4] Meyer UA. Pharmacogenetics and adverse drug reactions. Lancet, 2000; 356:1667.

[5] Tribut O, Lessard Y, Reymann JM, et al. Pharmacogenomics. Med Sci Monit, 2002, 8:RA152.

[6] Evans WE, McLeod HL. Pharmacogenomics - drug disposition, drug targets, and side effects. N Engl J Med, 2003, 348:538.

[7] McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. Annu Rev Pharmacol Toxicol, 2001, 41:101.

[8] Goldstein DB, Tate SK, Sisodiya SM. Pharmacogenetics goes genomic. Nat Rev Genet, 2003, 4:937.

[9] Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature, 2004, 429:464.

The life of Giuseppe Macciotta from: "Dizionario Biografico degli Italiani" Treccani

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Giuseppe Macciotta (Messina, 7 January 1892 - Cagliari, 31 August 1985) dedicated his work to the study of pathology present in Sardinia and the Mediterranean basin area, a prolonged period of observation. He graduated in medicine in May 1915, from which time, his military service finished, in 1919 he became an assistant in the Paediatric Clinic of Sassari. Giuseppe Macciotta stayed in this role until 1928 and then left Sardinia for only one academic year, that of 1928 - 1929, when he was entrusted with the management and teaching at the Paediatric Clinic of the University of Perugia and in fact, in the academic year 1929-1930, he directed the Paediatric Clinic of Cagliari. In this way, he was able to observe a great number of thalassemia cases, an illness which was very widespread in Sardinia.

From 1940 Giuseppe Macciotta identified and described a variant of thalassemia major (homozygote), whose symptoms include earlier onset and a more difficult course of illness. He defined this variant as sub-chronic erythroblastosis, referred to by many (including Roberto Corda) as Macciotta's disease. Subchronic erythroblastosis was characterised by appearance generally at the beginning of the second trimester of the baby's life, and a course of illness between 5 and 10 months and a fatal outcome.

The picture of the illness was dominated by hyperemolysis, erythroblastemia, medullar erythroblastosis and hyperbilirubinemia. The rapid course of the illness did not even allow time to damage the skeleton, and thus produced the formation of typical skeletal alterations

and cardiomegalia. This phenomenon has been observed by Macciotta in 45.21% of cases treated in the Cagliari clinic, with a slight prevalence of cases with male babies over female.

Thalassemia major (homozygote; Cooley's disease), on the other hand, was discovered in 51.4% of cases. The sub-chronic erythroblastosis (Macciotta's disease) was also identified by other researchers, including Gaetano Salvioli (1894-1982) and Marino Ortolani (1904 -1984).

In the years which followed, transfusional and precocious and rational therapies were carried out which permitted the abeyance of the debilitating course of the Macciotta's disease. The symptomatology was interrupted before any picture of greater or lesser seriousness could be drawn up.

Giuseppe Macciotta wrote various works (they are presented with the original title):

- Comportamento dei componenti proteici del siero di sangue in diverse condizioni nei bambini, in Studi Sassaresi, serie II I (1922), pp. 165-196;
- Contributo allo studio delle pleuriti mediastiniche nei bambini, Rivista di Clinica Pediatrica, XXIII, (1925);
- Contributo allo studio delle forme atipiche della malaria, ibid.;
- Sulla possibile influenza della malaria nelle eziopatogenesi del rachitismo, Il Policlinico, Sez. Medica, XIIIL (1927);
- Contributo allo studio delle meningiti eredoluoetiche Rivista di Clinica ed Igiene Infantile, III (1928);
- Rapporti fra malaria e tubercolosi nei bambini, Clinica ed Igiene Infantile, IV (1929);
- Contributo allo studio della terapia eterea nella pertosse, La Pediatria, XXXII (1924), pp.159-171;
- La proteinoterapia aspecifica nella cura della pertosse, ibid., pp.249-268;
- Contributo allo studio delle ricadute e delle recidive del morbillo; Il Policlinico, Sezione Pratica, XXXII (1925);
- Pleuriti sifilitiche in eredoluetici, La Pediatria, XXXIV (1926), pp. 1097-115;
- L'influenza e le sue complicanze nei bambini; La Clinica Pediatrica, IX (1927);
- Sèrums d'origine toxique et sèrums d'origine anatoxique dans la cure de la diphtèrie, Acta Pediatrica, vol. XI (1930);
- Alterazioni cardio-circolatorie e surrenali nel morbillo, La Clinica Pediatrica, XIII (1931);
- Sulla Roentegenterapia della meningite tubercolare, Scritti in onore di R. Jemma, a cura di I. Nasso e E. Schwarz, Milano, 1934, pp. 715-728;
- Neurassiti acute primitive di probabile origine virale nell'infanzia, Sardegna, Minerva Pediatrica, VIII (1956), pp. 432-8;
- Considerazioni e osservazioni sull'assetto proteico nella patogenesi delle varie forme di ricketiosi, La Clinica Pediatrica, VIL (1964), pp. 583-606;
- Studi e ricerche sperimentali sulla tetania. III) La funzione del timo nella patogenesi della tetania, Il Policlinico, Sez. Medica, XXXII (1925);
- Sui rapporti fra tetania ed avitaminosi da fattore B, La Clinica Pediatrica, XI (1929);
- Contributo clinico, etiopatogenetico ed anatomopatologico allo studio delle leucemie acute infantili, La Clinica Pediatrica, X (1928);
- Sulle leucemie congenite familiari, Rivista di Clinica Pediatrica, XXVI (1928);
- Aspetti reumatici e alterazioni osteoarticolari nella leucemia acuta nel bambino, Ras. Clin. Sci., XXXIII (1957), pp. 129-33;
- Recenti concetti patogenetici del favismo nei suoi vari aspetti, Ras. Clin. Sci., XXVIII (1952), pp. 106-7;
- Importanza di fattori costituzionali ed ambientali della origine di anemie eritroblastiche croniche (malattia di Cooley), subcroniche e forme minori e nella loro particolare frequenza in determinate regioni, Annali Italiani di Pediatria, II (1949), pp. 285-304;
- Eritroblastosi primitiva subcronica e suoi rapporti con la malattia di Cooley, ibid., IV (1951), pp. 179-189;
- Aspetti minimo ed a-eritremici della malattia di Cooley e della Eritroblastosi subcronica, ibid., V (1952), pp. 453-463;
- Aspetti del problema della malattia mediterranea, ibid., VII (1954), pp. 437-459;
- Rapporti fra atrofia del lattante e processi di senescenza, Minerva Medica, XXX (1964), pp. 728-36;
- Modificazioni giornaliere ed orarie nella eliminazione urinaria dell'acido urico, dell'urea e dell'azoto totale e modificazioni della uricemia in adulti sani in rapporto a diversi tipi di alimentazione Rassegna medica sarda, LCVI (1964), pp. 349-378;
- Direttive dietetiche nelle comunità infantili, Atti XIV Congresso nazionale di pediatria Firenze 23 al 26 settembre 1931 a cura di G.B. Allaria e A. Lucca, Varallo Sesia 1831,pp.11-70.

References

[1] Castiglioni A. Storia della medicina, II ed., Milano: Mondadori; 1948.

[2] Farnetani I, Farnetani F, Macciotta 's disease. Ped Med Chir 2005;27.

[3] Writings in honour of Giuseppe Macciotta in the 35th year of teaching, in Ital Ann Paediatr, 1963; suppl vol XVI :VII-XIII.

[4] Corda R. Necrologium. Ital J Paediatr1986;12:195.

[5] Bianco Silvestroni I. History of microcitemia in Italy, Roma; Fioriti; 2002:149.

[6] Schilirò G. Spigolature storiche sull'ematologia pediatrica in Italia, in: Andrea di Cataldo, Argomenti di ematologia, Catania 2001: 20.

[7] Farnetani I, Farnetani F. Ivo Nasso: infettivologo e fondatore della terapia intensiva neonatale. Minerva Pediatr 2006;58: 91-100.

[8] Farnetani I, Farnetani F. Giuseppe Macciotta: a paediatrician for Sardinia. Paediatric Notebooks. 2004;2:218.

[9] Latronico N. Storia della pediatria, Torino: Minerva Medica 1977: 672-674.

[10] Panorama biografico degli italiani di oggi, Roma: Curcio 1956; II: 899.

Vaccination perspectives

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Vaccination represents one of the major tools in public health policy. The contribution of efficient and safe vaccines to the improvement in welfare of mankind is comparable only with the availability of drinking water. The benefit of vaccination is not limited

to vaccinees, it also impacts on the non vaccinated cohorts for the "herd immunity" effect which interrupts the infection transmission and reduces the risk of exposure to pathogens. The herd immunity led to smallpox eradication worldwide in 1980 and to polio eradication from the European region in 2002, with the last reported case in Turkey in 1998. However the risk of re-emergence remains with the hazard of importing poliovirus from endemic areas.

In Italy, despite the successful results of universal vaccination and the consequent dramatic decrease of diseases like diphtheria and tetanus, the vaccine coverage rates against some important infections are still different through the regional areas. One of the major target in national and regional plans was the satisfactory vaccination coverage and the disappearance of some preventable diseases like measles and rubella. Nevertheless, national surveillance data report coverage rates of 95% in vaccinations enforced by the national program during the first year of life (diphtheria, tetanus, polio, pertussis, *Haemophilus influenzae* B, hepatitis B) and of 85% in those administered in the second year of life (measles, rubella and mumps). Paradoxically, while the incidence of these infections decreases, the safety and the benefit of vaccines is increasingly questioned by some groups of citizen.

The European week on vaccination, held for the first time by 9 countries in 2005, has been promoted this year by 25 countries, mostly of the past Soviet Union, representing 57% of the whole European population. Italy was represented also by the autonomous Bolzano region where the vaccination policy is particularly difficult due to the presence of local anti-vaccination lobbies. The event was co-ordinated by the WHO office for Europe and it was also supported by important regional partners as UNICEF and the European Center for Disease Prevention and Control (ECDC).

Despite evidence that in the European region vaccines have controlled highly contagious or fatal infectious diseases, the coverage rates of vaccination must remain high to prevent the re-emergence of tetanus, diphtheria, measles or polio which cause deaths, disability and enormous costs for health systems.

Annually 32,000 children are killed by preventable diseases in the European region, where over 500,000 children are still unvaccinated and are thus potentially susceptible to infections.

The WHO aim to eradicate polio and measles and congenital rubeola has been an important reference in Europe; measles incidence has dramatically decreased in the last 10 years due to the implementation of the two-dose schedule. Nevertheless measles elimination has not been achieved and only a few countries succeeded in containing incidence level below 1 case over 1 million during recent years.

Although measles decreases, in some countries a re-emergence of the infection was observed: in Poland (1988), the Netherlands (1999-2000), Spain (2001-2002) and Latvia (2002). During the last two years outbreaks of measles were reported and 55,000 cases were registered in 2006. Thus EU countries have continuously to face outbreaks, which may exceed the national borders.

The success of vaccination strategy is not always linked to socioeconomic level: high income countries which consistently invest in the healthcare field may have vaccine coverage rates lower than those reported by poorer countries and have higher incidence of preventable diseases. The reason for low vaccine coverage rates is the absence of the disease leading to underestimation of the risk, just as the dramatic decline of outbreaks during recent years has led to considering many important infectious diseases as a recall of the past in some European countries.

The confidence in vaccines can be compromised by concern about adverse reactions. The vaccination programs could thus become paradoxical victims of their success. For this reason it is of paramount importance to provide correct and evidence based information about vaccine safety and this represents one of the major objectives of the EU. The EU funds specific programmes such as: 1) *Venice* (Vaccine European New Integrated Collaboration Effort), which aims to spread knowledge and best practice about vaccines and to improve cooperation among European countries; 2) *Euvac.Net*, aimed at creating a surveillance network and laboratory references throughout Europe about vaccine preventable diseases (measles, rubella, congenital rubella, mumps, varicella, pertussis); 3) *Vacsat* which aims at improving vaccination programs devoted to the study of the attitude to vaccines and the training of health care workers.

The triennial Italian vaccine plan 2005-2007 (G.U., Suppl Ord 14 April 2005) provided the new calendar of compulsory and recommended vaccinations, considering also new vaccines available in Italy such as varicella, C meningococcus and pneumococcus vaccines. In the second half of 2006 the European Agency for Drugs (EMEA) licensed two vaccines against rotavirus and one vaccine against human papillomavirus (HPV); a second HPV vaccine is under evaluation by EMEA and will probably be licensed at the end of this year. The EU countries have to decide if and how to introduce these new vaccines in national programmes, after evaluating specific epidemiological and economic analysis. The VENICE study investigated about the decisional processes in the different participating EU countries (all except Malta and Estonia for rotavirus vaccine) and Island and Norway. Preliminary results report some information. After March 4, Austria, France, Germany and Italy planned to include HPV vaccination in their national programmes. The target populations are 12-year old girls in Italy, 14-year old girls in France where the vaccine is also recommended in sexually active women until 23 years of age, girls between 12 and 17 years of age in Germany, all girls before sexual activity in Austria but vaccination in both sexes is considered. The HPV vaccine will be provided free of charge to the target population in Italy while in the other three countries the issue is under evaluation.

The expert advisory committee of Greece and Slovakia recommended to include HPV vaccination in the national programmes but no official decision has been taken yet. The national immunization advisory boards of nine countries are still considering the problem; seven countries referred to face the problem in the future while in five countries the question is not considered so far.

With regard to the rotavirus vaccination, five countries have discussed the problem until March 2007. Austria, Belgium and Luxemburg have included the vaccination in the national immunization programmes. The

expert advisory committee of Slovakia recommended to introduce the vaccine in the national immunization programme but no official decision has been taken so far. France, Germany and Spain did not to recommend the universal vaccination in newborns. In Poland the decision is on discussion and in 11 countries the issue is planned for the future; nine countries did not consider the problem yet.

The *Venice* study is the first research monitoring the decisional process for the introduction of new vaccines in the national European programmes. Considering that only a few countries have introduced universal HPV and/or rotavirus vaccinations, it is evident that the issue is very complex. Further analysis and the follow-up of the situation are needed to identify the main obstacles to the introduction of new vaccines in the universal programmes of immunization.

References

Eurosurveillance Weekly, vol. 10, n. 4 - April 26, 2007
http://venice.cineca.org
http://www. epicentro.iss
http://www. ecdc.europa.eu
http://www. europa.eu

News on pharmacotherapy of acute diarrhea

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Summary

The aim of this presentation is to review the current literature, focusing on the therapeutic approach to acute diarrhea in pediatrics.

Acute diarrhea

Acute diarrhea is one of the most important life-threatening conditions for children. Every year 1 billion of pediatric cases are reported and 2 millions of children < 5 years old, particularly in developing countries, die. In countries with high-level health organizations a dramatic decrease of mortality was obtained, although acute diarrhea is still associated with high morbidity. For example in the United States diarrhea is responsible for 220,000 hospital admissions in children < 5 years old,

About 70% of acute diarrhea is caused by virus, particularly Rotavirus, 15% by bacteria. Major complication is represented by dehydration. Thus, prevention of dehydration and optimal rehydration are the major goals of management.

Nowadays, pharmacologic options of acute diarrhea are limited to probiotics and antibiotics. On the contrary intestinal mobility inhibitors, such as loperamide, are contra-indicated.

In recent years the new antidiarrheal drug racecadotril has been developed, with strong antisecretory activity, rapidly hydrolized in its active metabolite, tiorfan, which inhibits encephalinase. The inhibition of this enzyme prevents the inactivation of endogenous encephalins, released by submucosal and mesenteric neurons, and prolongs their physiological actions. The encephalins are neurotransmitters of the gastrointestinal tract that mediate their effect with the activation of δ-oppiaceous receptors and consequent selective increase of chloride absorption, inhibiting AMP cyclic. The result is a reduction of water and electrolyte secretion without gut motility alterations. Moreover racecadotril action is present only in conditions of hypersecretion and does not have effects on basal secretory activity. With an oral administration of 1.5 mg/Kg of racecadotril, the inhibition of plasmatic encephalinase starts after 30 minutes, while peak (90%) is reached after 2 hours. The inhibition of plasmatic encephalinase remains for about 8 hours and half-life is about 3 hours, but these are dose-dependent effects. 90% of tiorfan is bound to plasmatic proteins and only 1% of administered dose distributes into tissues. Then the drug is transformed as non active metabolites, which are eliminated mainly with urines.

Many studies are available in pediatrics. In a population of children 3 months to 3 years old affected by acute diarrhea without blood and without significant dehydration it was demonstrated a reduction in the number of episodes in the first 48 hours (6.8 vs 9.5; $p<0.001$), duration of diarrhea (97.2 vs 137.7 h; $p<0.001$) and less frequent medical consultation (18.4% vs 34.6%; $p<0.05$) [1].

Similarly, in another study fecal output in the first 48 hours with racecadotril was 46% lower (92 g/kg vs 170 g/kg; $p<0.001$) if compared with placebo [2]. Total output was 53% lower than placebo. Mean duration of diarrhea in children treated with racecadotril, either with positive either with negative rotavirus tests, was 52-72 h. Furthermore the drug allowed a 1/3 reduction of rehydration solution (439 ml vs 658 ml in the first day e 414 ml vs 640 ml in the second day).

Similar results were reported by other authors. Moreover a urinary Na/K <1 was observed in 1/5 in racecadotril treated, compared in 1/2 of control, suggesting a better hydration in the former group [3].

Recommended dosages in published studies are 1.5 mg/Kg every 8 hours for 5 days for a maximum of 7 days. The drug is not utilized for bloody diarrhea or antibiotic-induced diarrhea.

Side effects frequency is similar to the placebo group (vomiting 5.2% and fever 2.2%). Headache, hypokalemia, paralytic ileus, skin rashes and bronchoconstriction have been reported only very rarely.

Finally, experimental studies have demonstrated that racecadotril does not promote bacterial growth in the gut and does not pass in the CNS after oral administration. Thus the drug is not associated with neurotoxic effects.

References

[1] Cojocaru B, Bocquet N, Timsit S et al. Effect of racecadotril in the management of acute diarrhea in infant and children. Arch Pediatr 2002; 9: 774-9.

[2] Salazar-Lindo E, Santisteban-Ponce J, Chea-Woo E et al. Racecadotril in the treatment of acute watery diarrea in children. N Engl J Med 2000; 343: 463-7.

[3] Cezard JP, Duhamel JF, Meyer M et al. Efficacy and tol-

erability of racecadotril in acute diarrhea in childen. Gastroenterology 2001; 120: 799-805.

[4] Caramia G, Ruffini E, Fanos V. Infezioni e Farmacoterapia Pediatrica. Biomedia Editore 2007.

News about Crisponi syndrome

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Crisponi syndrome (CS) (MIM 601378) is a severe rare autosomal recessive condition described for the first time in 1996 by Giangiorgio Crisponi on 17 patients of 12 Sardinian families, characterized by facial muscle contraction, dysmorphic features, camptodactyly, hyperthermia, sudden death [1]. Recently Accorsi et al [2] and Nannenberg et al [3] reported each a further case with the same phenotype and suggested to name this syndrome after the original contributor. The disorder is evident at birth and characterized by contraction of facial muscles elicited by tactile stimuli or during the crying, with trismus and abundant salivation simulating a tetanic spasm. Also, contractions of the oro-pharyngeal muscles accompanied by apnea, hypopnea, cianosyis associated with the absence of the swallowing reflex are observed. These contractions slowly disappear as the infant calms. Dysmorphic features such as large round face, chubby cheeks, broad nose with anteverted nostrils, long philtrum, micrognathia and bilateral camptodactyly are present in all the patients. The clinical course is characterized by marked suckling and swallowing difficulties requiring nasogastric tube feeding. In the course of the disease these patients present hyperthermia with core temperatures of about 38°C, associated with irregular febrile episodes over 42°C, with onset ranging from birth to a few weeks. In some patients generalized seizures occur. Most patients died within the first months of life during hyperthermic episodes. In two patients, reduced levels of gamma butyric acid in cerebrospinal fluid were noted, but the pathogenic relevance of this finding is unclear. Up till now only five Sardinian patients are still alive. In the rare surviving patients, hyperexcitability slowly disappears in the first years of life, such as febrile episodes. All survivors develop a severe progressive kyphoscoliosis requiring corset therapy or corrective surgery. We also noted paradoxical sweating after exposure to low ambient temperature in some affected adolescents.

We performed homozygosity mapping in five Sardinian and three Turkish families with Crisponi syndrome using high-density SNP arrays and identified a critical region on chromosome 19p12-13.1. The most prominent candidate gene was *CRLF1*, recently found to be involved in the pathogenesis of Cold induced Sweating Syndrome type 1 (CSS1) [4,5]. CSS1 belongs to a group of conditions with overlapping phenotypes (camptodactyly, feeding difficulties, elbow contractures, scoliosis and cold induced sweating), also including Cold induced Sweating Syndrome type 2 (CSS2) caused by mutations in the Cardiotrophin-like Cytokine Factor 1 gene (*CLCF1*), Stuve-Wiedemann syndrome (SWS) Schwartz-Jampel Syndrome type 2 (SJS2) caused by mutations in the Leukemia Inhibitory Factor Receptor (*LIFR*) gene. All these syndromes are caused by mutations of genes of the Ciliary Neurotrophic Factor (CNTF) receptor pathway, which is known to be involved in neuromotor survival. Here we describe the identifications of four different *CRLF1* mutations in eight different CS families including a missense mutation, a single nucleotide insertion, a nonsense and an insertion/deletion (indel) mutation, all segregating with the disease trait in the families. Comparison of the mutation spectra of CS and CSS1 suggests that neither the type nor location of the *CRLF1* mutations point to a phenotype/genotype correlation that would account for the most severe phenotype in the CS. Our findings provide further evidence for the importance of the CNTF receptor pathway in the development of autonomic and motor functions of the nervous system.

References

[1] Crisponi G. Autosomal recessive disorder with muscle contractions resembling neonatal tetanus, characteristic face, camptodactyly, hyperthermia, and sudden death: a new syndrome? Am J Med Genet 1996; 62:365-71

[2] Accorsi P, Giordano I, Favarelli F. Crisponi Syndrome: report of a further patient, Am J Med Genet 2003; 123:183.185

[3] Nannenberg E, BijmerR, Van Geel B, Hennekam R. Neonatal paroxysmal trismus and camptodactyly: the Crisponi syndrome. Am J Med Genet 2005; 133:90-92

[4] Crisponi L, Crisponi G, Meloni A, et al. Crisponi Syndrome is caused by mutations in the *CRLF1* gene and is allelic to cold-induced sweating syndrome. Am J Hum Genet 2007; 80:971-81.

[5] Dagoneau N, Bellais S, Blanchet P, Sarda P, Al-Gazali LI, Di Rocco M, et al. Mutations in cytokine receptor-like factor 1 (*CRLF1*) account for both Crisponi and cold-induced sweating syndromes. Am J Hum Genet. 2007; 80:966-70.

Pediatrics in the third millennium

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"Pediatrics is not the study of a single organ or apparatus, like other branches, thus it is not a secondary or complementary medicine..., but examines the whole organism during the very special and brief time of development, with phases of very active evolution". Francesco Fede, 4th Congress Italian Society of Pediatrics, Firenze 1901.

The main goals of pediatrics in the third millennium

Integrated multidisciplinary approach: the future of human society relies on the well-being of children and on the possibility to offer to developing organisms optimal physical and psychological growth. In this way it is well recognized nowadays the importance of supporting families in promoting children to develop their potential at maximum level.

Social changes determined by communication technology, media power, new family dynamics, research evolution, including new drugs development, require new answers to the new needs in pediatric care, from birth to the adult age.

Improvement of health workers and services: Main

area of interventions are represented by: heath sanitary systems and sanitary services, clinical research, guidelines creation and revision, professional training, essential drugs availability, attention to antimicrobial resistance problems, management of new pathologies like HIV, development and maintenance of quality of assistance.

Improvement of parental care: Pediatricians must learn to help parents, namely communities of immigrants, identifying clinical signs of severe illness. Moreover Pediatricians should favor perinatal care and vaccinations, improve home assistance, sustain breast feeding until 6 months of life, avoid exposure to tuberculosis and malaria, stimulate adequate physical and psychological growth of their little patients.

Planning of interventions and epidemiologic studies: Application of epidemiologic studies in programming health strategies, including evaluation of costs and cost/efficacy ratio, is considered a correct approach.

In developing countries 10 millions of children <5 years old die each year. Pneumonia, diarrhea, malaria, measles and malnutrition are examples of illness that could and must be prevented.

WHO data demonstrate high mortality in Africa (25.7% in Angola, 17.8% in Malawi), or in Asia (26.3% in Afghanistan) due to war and related problems. In Europe, including France and Italy, mortality is 0.5%, although interventions are needed in countries with higher ratios (Albany and Romania 2%).

Care and cure of illness: Many diseases of the newborns and infants are preventable today and early diagnosis and appropriate therapeutic intervention can significantly reduce infant mortality.

Vaccinations have lead to excellent results in terms of infectious disease prevention. Many diseases can be treated at home or with hospital day regimen.

Hygienic rules and rehydration, new antidiarrheal drugs and vaccines against rotavirus have greatly improved diarrhea prevention and treatment.

Similarly malaria can be prevented with protective nets and adequately managed with drug prophylaxis and treatment.

Malnutrition in developing countries is still the main cause of mortality; on the contrary wrong dietary habits are related to obesity in industrial countries.

Adolescence: Most of adolescent become adults without problems. However, in this period of life problems can be represented by sexual illness, drug abuse, behavior alterations, including accidents, abuses and suicides. In the world other cause of death are malaria and tuberculosis.

Different social and economic factors, even in industrial countries, have increased adolescent discomfort. It is necessary to favor a correct sexual education of adolescents, to sustain early pregnancies, and to inform about sexual transmitted diseases.

HIV: The WHO reports that more than 4 millions of children <15 years old were infected by the start of epidemia (90% of cases by materno-fetal transmission); moreover more than 10 millions of young people (15-24 years old) are infected by HIV. Consequently maximal efforts should be directed to prevent neonatal infection, to ameliorate the cure of infected children, and to improve the prevention and the management of adolescent with HIV.

Antibiotic resistance: This is an extremely important health problem. The inappropriate use of antibiotics makes easy antibiotic resistance and minimize a therapeutic rationale.

Nutrition: A correct early nutrition from early stages of life, namely from the fetus to the adult, allows a correct growth. Information of families and adolescents on correct dietary habits is mandatory.

The role of the Italian Society of Pediatrics (ISP)

ISP was founded in Turin in October 1898 and the first president was Francesco Fede until 1905. ISP it is not only an association of professional men and women (8000 fellows), but it is mainly a scientific society interested in research, formation and updating. The latter includes emanation of guidelines, consensus conferences on emerging clinical problems, promotion and governance of the scientific production, communication on new developments to the families and to the teachers.

The principal goals of ISP refer to improve the net between institutions, regional sections, the integrated management of patients with specialized branches of pediatrics, such as neurosurgery, pediatric cardiology, orthopedics. Furthermore ISP promotes the introduction of new techniques of updating, together with the Schools of Specialization in Pediatrics.

Strategic tools of the executive board member are: research observatory, epidemiologic observatory, risk management and clinical risk, guidelines, ethics, deontology and revision of the statute.

Preliminary data presented at the Pisa Forum show that in the next 10 years the number of pediatricians in Italy could not be sufficient to preserve the present model of assistance, namely the family pediatrician and the hospital pediatrics.

In the last years there is an increase of migration of pediatricians from the hospital to the territory, with lacks in intensive care units.

The new formula suggested by the Ministry of Health and called "Unit of Primary Care" could be a possible solution to the problem.

Examples of relevant health problems are: screening, obesity, computerized support to epidemiology, vaccination, breastfeeding, alcohol dependence in emergency rooms, prevention of accidents in the home, vaccinations, off-label and unlicensed drugs, clinical trials in pediatrics.

Urinary tract infections in infants and children: clinical findings and diagnosis

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Introduction

Up to date urinary tract infections (UTIs) are the most common serious bacterial infections in infants and young children. UTI is defined as the presence of bacteria in urine along with symptoms of infection. The

incidence of UTI in infants ranges from approximately 0.1 to 2.0 percent in all newborn infants to as high as 20 percent in preterm newly born infans and neonatal at risk population (i.e. low-birth-weight infants) as experienced by Cataldi *et al* [1-2].

Before age one UTIs occurs more frequently in boys than in girls. After age one, both bacteriuria and UTI are more common in girls.

In preschool-age children, the prevalence of asymptomatic infections diagnosed by suprapubic aspiration in girls is 0.8 percent, compared with 0.2 percent in boys. The UTI may be limited to the bladder, one or both kidneys, or they can involve both sites. [3]

In general, infections of the bladder (cystitis), although they cause substantial morbidity, are not regarded as serious bacterial infections. By contrast, infections that involve the kidney (pyelonephritis) may cause both acute morbidity and lead to scarring with the consequences of hypertension, preeclampsia, and chronic renal disease. [4]

Caregivers need of course to pay attention to the site of infection.

History and clinical findings

The spectrum of illness extends from minor symptoms to life-threatening systemic illness.

The diagnosis of UTI may be suggested by certain signs and symptoms, mainly aspecific in the newly born infants as fever, prolonged jaundice, poor feeding, failure to thrive and severe systemic illness. Along the first year of life fever, poor feeding, vomiting, diarrhoea. Fever may be mild or absent. Children with UTI frequently have symptoms as fever, dysuria, frequency, urgency, nocturia, haematuria, cloudy or foul-smelling urine, suprapubic discomfort or tenderness or secondary incontinence [3-5].

Neonates and infants aged 0-2 months who have pyelonephritis usually do not have symptoms localized to the urinary tract. UTI is discovered as part of an evaluation for neonatal sepsis.

Infants and children aged 2 months to 2 years with pyelonephritis may have a history of unexplained fever (T>38°C). High temperature (>38.5°C) is not indicating absolutely pyelonephritis, because proof of upper urinary tract infection (UTI) requires imaging to demonstrate new lesions in renal tissue.

These patients are at higher risk for renal injury than are older children, possibly because the lack of localizing signs of infection delays the start of antimicrobial drugs: so an infant or child with unexplained fever for more than 3 days should be submitted to a urine analysis (and cultural examination).

Some infants with pyelonephritis in the first two years of age may have fever and few other symptoms, whereas others are acutely ill, a history of irritability, decreased oral intake, abdominal pain, vomiting, and loose bowel movements.

Children aged 1-2 years may present with voiding symptoms suggestive of cystitis, with crying on urination or only a foul odor to the urine in the absence of clinically significant fever (temperature <38°C).

Children aged 2-6 years, with febrile UTI (pyelonephritis) usually have systemic symptoms with loss of appetite; irritability; and abdominal, flank, or back pain. Voiding symptoms may be present or absent [5].

Children with acute cystitis have voiding symptoms with little or no temperature elevation. Voiding dysfunction may include urgency, frequency, hesitancy, dysuria, or urinary incontinence.

Children older than 6 years and adolescents: in this age range UTI usually affects the lower tract, but pyelonephritis also occurs. Symptoms are similar to those in children aged 2-6 years.

Girls who have pyelonephritis in infancy or early childhood, including those with persistence of vesicoureteric reflux (VUR), usually have cystitis with UTI when they are older. They are also prone to have a recurrence during pregnancy.

Infants and younger children with pyelonephritis usually have no localizing findings, but they are febrile and irritable [3-5].

Laboratory diagnosis

Usually the gold standard for the diagnosis of UTI is culture of the urine: detection of more than 10^5 organisms per millilitre of suitably collected urine. Urine cultures that grow multiple organisms usually indicate contamination or multiple urinary calculi. If the urine is collected under sterile conditions (e.g. suprapubic aspiration, or 'in-and-out' catheterization) colony counts as low as 10^2 to 10^4 per millilitre may indicate infection.

In the absence of clear evidence, pad/nappy or bag may be used for collecting urine samples from non-toilet trained children.

The evidence suggests that CVU samples had similar accuracy to suprapubic aspiration (SPA) samples when cultured, and as CVU is a non-invasive collection method that can be employed in the general practitioner's surgery, this was chosen for the algorithm.

Because culture results are not available for at least 24 hours, there has been considerable interest in evaluating tests that may predict the results of the urine culture so that appropriate therapy can be initiated at the first encounter with the symptomatic patient. The tests that have received the most attention are urine microscopy for leukocytes (WBC) and bacteria, and biochemical analyses for leukocyte esterase and nitrite that can be assessed rapidly by dipstick [5]. A dipstick test which is positive for both nitrite and leucocyte esterase (LE) indicates a very high likelihood of a UTI. Dipstick negative for LE and nitrite or microscopic analysis negative for pyuria and bacteriuria of a clean voided urine (CVU), bag or nappy/pad specimen can be used to rule out UTI, avoiding the need for further investigation for UTI. It is not possible to define further which clinical signs and symptoms should inform the decision to test for UTI [4-5].

A few years ago Gorelick and Shaw concluded that pyuria of at least 10 WBC/hpf or at least 10 WBC/mm^3 and bacteriuria (any) are best suited for assessing the risk of UTI in children. Their results were updated by Huicho et al. through a meta-analysis in order to determine the risk of UTI in children [5].

In recent years serum procalcitonin (PCT), a marker of bacterial disease, has been tested to predict the level of infection in children with UTI. Procalcitonin seems to be a valid biological marker, with an acceptable sensitivity and specificity, which predicts a renal involvement of the infection (pyelonephritis), in comparison with the low specificity of C-reactive protein. Procalcitonin also seems to be correlated with the degree of the involvement at the moment of diagnosis of febrile urinary tract infections and with scarring.

Pecile et al suggest this measurement could be useful for the treatment of children with febrile UTIs, allowing prediction of patients at risk of permanent parenchymal renal lesions. Although the number of studies published are limited, procalcitonin has, however, demonstrated some specific characteristics that make it more reliable than CRP in highlighting renal lesions during UTIs. These are, firstly, the velocity with which it is induced by the infectious stimulus, which increases somehow its high negative predictive value; second, the better specificity compared with CRP in detecting renal involvement during febrile UTIs; and lastly, but maybe the most interestingly, even though documented in only two studies, the progressive increase of its blood concentration with the increase of the renal lesion's entity. In conclusion, for the time being, procalcitonin can be considered an accurate and sufficiently reliable new biological marker to be used in clinical treatment of febrile UTIs. [6]

Imaging and UTI

Routine imaging for children aged 2 years or more with an initial UTI is not recommended. For children under 2 there is no firm evidence base.

All children aged 2-5 with an initial UTI should be monitored and investigated further if they experience a second UTI.

Imaging studies are the standard of care for young children with a first UTI. It still to be established the exact role the value of routine imaging studies after the diagnosis of a first febrile UTI in 309 children 1 to 24 months of age. Renal ultrasonography, DMSA, and contrast voiding cystourethrography (VCUG) were performed in almost all of the children. Of the 309 ultrasonograms, 272 (88%) were abnormal and 12 showed modest abnormalities that did not alter treatment.

Further imaging investigations

A test for the localisation of UTI as an initial step in the investigation of these children would allow the exclusion of all children with a lower UTI from further investigation.

Acute Tc-99m-DMSA (dimercaptosuccinic acid) scintigraphy remains the reference standard for the localisation of UTI. These scans are costly, invasive, and incur a radiation load. A noninvasive test would be desirable.

Most investigators agree that further research is required regarding the accuracy of ultrasound in diagnosing underlying abnormalities, and its impact on patient outcome. There is insufficient evidence to recommend any further investigation routinely: in the absence of any effect on patient outcome, universal imaging (for example, voiding cystourethrography (VCUG) for reflux or DMSA scintigraphy for renal scarring) cannot be justified. The decision on whether or not to perform these examinations should be made on an individual patient basis. Actually many Authors suggests that VCUG should be reserved for those children who have been deemed to require further investigation and the DMSA scan is abnormal or the ultrasound has shown an abnormal bladder. [4,5]

In conclusion further research concerning the effects of these imaging techniques on long term patient outcome is required [7].

References

[1] Cataldi L, Fanos V. Urinary tract infections in the newborn: diagnosis and therapy. In: Neonatal Nephrology in Progress Cataldi L, Fanos V, Simeoni U (eds) Agorà Ed Lecce 1996: 183-198.

[2] Cataldi L, Salvaggio E, Prota M. L'infection urinaire du prématuré: existe-t-elle? Progrès en Néonatologie 1990.

[3] Klein JO, Long SS. Bacterial infections of the urinary tract. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 4th ed. Philadelphia: Saunders, 1995:925-34.

[4] Wald E. Urinary tract infections in infants and children: a comprehensive overview. Curr Opin Pediatr 2004;16:85-8.

[5] Cataldi L, Fanos V. Urinary tract infections in infants and children: prophylaxis and therapy. Proc. 1st International Workshop of Neonatology Cagliari 24-25 Oct 2004.

[6] Pecile P, Romanello C. Procalcitonin and pyelonephritis in children. Curr Opin Infect Dis 2007;20(1):83-7.

[7] Fanos V, Cataldi L. Antibiotics or surgery for vesicoureteric reflux in children. Lancet. 2004 Nov 6-12; 364 (9446): 1720-2.

Lower urinary tract symptoms (LUTS) and recurrent urinary tract infections (UTIs) in children

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Introduction

Urinary tract infection (UTI) is the most common serious bacterial infection in infants and children. Early detection and timely treatment of UTIs are important to prevent progression of infection to pyelonephritis and to avoid late damage such as renal scarring or renal failure.

Children with lower urinary tract symptoms (LUTS) are widely represented in the school age population (15-25%). Children with LUTS have multiple synergistic risk factors that predispose them to recurrent UTI.[1] In the anatomically and neurologically normal child, LUTS are usually caused by persistence of incorrect toilet training, an important contributor to recurrent urinary tract infections (UTI). Obstruction and dysfunction are among the most common causes of urinary infection.[2] Children with LUTS may present combinations of incontinence, recurrent UTI, and constipation.[1,3] Usually, the ordinary therapy for recurrent UTI is based on the use of antibiotic prophylaxis.

A simple behavioural approach, with the application of "urodynamic" tools, could reduce the recurrence of UTI. Since LUTS are very common in the school age population this step-wise approach results in considerable savings and avoids more demanding diagnostic procedures in a large proportion of children.

Definitions

According to the International Consultation on Incontinence (ICI), the new definitions of LUTS, lower urinary tract dysfunction (LUTD) and observed urodynamics are compatible with codification systems and universal classifications such as the World Health Organization's International classification on Functioning, Disability and Healthy (ICHDH-2) and the

International Classification of Disease (ICD-10).[4] LUTS are not unique to any given pathology and are not a decisive element in the formulation of a diagnosis due to the fact that they may coexist with a variety of pathological conditions (e.g. urinary tract infection).

Physiology of voiding in childhood

Normal bladder storage is regulated by a low-pressure detrusor adaptation mechanism. Voiding takes place when a detrusor contraction and the complete relaxation of the sphincter occur contemporaneously. This process requires normal detrusor sensitivity and adequate resistance to the emptying of the bladder.

The neurophysiologic mechanisms involved in the control and coordination of normal bladder function, are comprised by an integrated effort of the sympathetic and parasympathetic nervous system, as well as somatic innervation, which are ultimately controlled by the upper nervous centers.

The sensation of a full bladder develops between the first and second year of life. The ability to voluntarily initiate and interrupt voiding at any point of bladder storage is developed between the second and third years of life. Central inhibition is crucial in order to achieve continence. During this period, there is a progressive development toward a socially conscious continence and more adequate control of voiding.

Generally by age 3-4, a child remains dry both day and night. The child has learned to inhibit the micturition reflex and to postpone voiding in ways and places that are socially acceptable. This is followed on average with 4-6 voidings during a 24 hour period.

A variety of dysfunctions of the detrusor-sphincter-perineal complex may develop during the various developmental stages of the micturition control mechanism.

Pathogenesis

Physiologic factors such as dysfunctional voiding and constipation place children at risk for developing urinary tract infections. Infrequent voiding and incomplete bladder emptying could lead to the development of a UTI. McKenna and associates observed that the most common pattern in children with voiding dysfunction is pelvic floor hyperactivity.[1]

Constipation is also an associated factor in the development of a UTI. If stool is chronically in the rectum, more bacteria tend to colonize in the perineum, increasing the risk of UTI.[1] Factors that predispose to recurrent UTI with voiding dysfunction could be: abnormal pelvic floor contraction with voiding, anatomic bladder abnormalities (trabeculation, diverticula), vesicoureteric reflux, ectopic ureter constipation, elevated residual urine and high pressure voiding. [4,5]

Clinical features

Control of voiding in children is closely tied not only to a social context, but also to biological factors, as well as physical and emotional development.

A considerable portion (15-25 %) of the childhood population is affected by LUTS.

It can be stated that the majority of young patients seen by a paediatrician for LUTS fall into two groups:

- Girls age 4 and up who demonstrate "retentionist" tendencies frequently accompanied by recurrent UTIs.
- Children of both genders of preschool age with frequent daytime voiding without signs of infection.

In our Institution we propose a complete non-invasive integrated urodynamic study with the preliminary use of a weeklong frequency-volume chart (FVC), to start a programme of voiding re-education in order to avoid recurrent UTI. This approach offers diagnostic results that have been confirmed by subsequent urodynamic studies, therefore providing a useful screening method for children with LUTS and recurrent UTIs. Moreover, the application of the FVC itself is an important tool in the treatment of wetting disorders in children.

Resolution of constipation is crucial because it is directly correlated with significant reduction of UTIs and improved voiding patterns.[6]

Diagnosis

Ideally, procedures to be used in the evaluation of voiding dysfunction in children should be non-invasive, reliable and reproducible.

Urodynamic studies which include one invasive and one non-invasive approach are of important significance in the diagnosis of LUTS.[7]

Non-invasive urodynamics consist both in a clinical (voiding history, bladder diary and questionnaires) and instrumental (urinary flowmetry, EMG with surface electrodes, ultrasound).

History-taking furnishes information regarding stages of development, mental state, voiding frequency (on average 6-7 times in a 24-hour period), voided volumes, the type and severity of urinary symptoms and even the possible presence of constipation (normal evacuation frequency in preschool age: 1-2 times in 24 hour period).

The patient's examination identifies possible markers of neurological pathologies (skin discoloration in the sacral-coccygeal region, angiomas, lipomas, hair growth, "dimples", and anomalies of the intergluteal cleft).

The compilation of questionnaires and diaries are essential: the voiding frequency chart, in which each micturition in a 24-hour period is listed, the frequency-volume chart, where the time and volume of each voiding are recorded, and the bladder diary which offers the most complete measurement by recording schedules, volumes, episodes of incontinence, absorbent pad usage, volume of fluid collected, severity of incontinence and urgency.

A more exhaustive bladder diary in which the child scores urgency to void on a scale of 1 to 3 (from initial urge to most urgent) should be used when the socio-cultural state of the patient and his/her family permits it.

The first line of instrumental studies consists in: renal ultrasonography, urinary flowmetry in conjunction with possible surface electrode EMG.

Ultrasound combined possibly with urinary flowmetry allows for a morphological study to be carried out measuring the density and intensity of the reflection of the bladder wall, the non-invasive measurement of volume, the possible presence of a dilated ureter, diverticula, ureteroceles, etc.

The study is complete once an evaluation is made of the post-void residual. In most cases, this is the final step in the diagnosis of a patient. This integrated, non-invasive urodynamic study provides results that can and should be confirmed by invasive diagnostic measures.

The diagnostic framework established by the

International Consultation on Incontinence (ICI) emphasizes that the application of invasive instrumental diagnostics should be reserved to a limited portion of the patient population.

Therapeutic considerations

The approach to LUTS and recurrent UTI in children is based on the use of the bladder diary in conjunction with that of a weeklong frequency-volume chart (toilet re-training) [8], constipation treatment, an increase in fluid intake, pelvic floor retraining and the initiation of prophylactic antimicrobial if breakthrough UTI develops.

The behavioural approach using the frequency-volume chart can be applied by both family and clinical staff by means of positive reinforcement.

Materials and methods

In our institution, we studied 184 patients (101 females and 83 males) ages 3 to 13 (mean age 7.2 years) with LUTS referred to our hospital between January 2002 and January 2007. A history of recurrent UTIs was present in 63 of the children (34.2%) with LUTS. A psychologist and a paediatric urologist evaluated all children. Sessions typically lasted 45 minutes. A complete history with specific emphasis on voiding patterns and physical examination (abdominal, pelvic, perineal and neurological) were done and urinalysis was performed in all cases. All patients were trained to use a weekly FVC and evaluated again after 15, 30 and 45 days for the assessment of voiding behaviour of the child. The FVC was also utilized in the evaluation as a positive behavioural reinforcement tool.

In selected cases, where the integrated non-invasive approach raised suspicion for pathological dysfunction, (visible ureters at US, high bladder volume, presence of high post residual volume) the study was completed with a kidney ultrasound, invasive urodynamic exams and/or cystoscopy.

In order to give children correct information about "toilet training", a simple behavioural approach along with a psychologist was initiated in all patients.

Therefore a programme of voiding re-education was proposed, with a system of incentives that direct the child toward a result of the desired behaviour. Patients are thus trained to use FVC frequently.

Results

Among the 184 children with LUTS considered in this study, 167 (90.7%) showed an improvement of symptoms during the programme of voiding re-education; of the 63 children with recurrent UTI, 55 (87%) had a complete lack of UTI in 1 year follow-up. 11 children (6%) underwent invasive urodynamic evaluation and eventually MRI of the spine. In eight (6 females and 2 males) of these cases, this procedure revealed serious urological abnormalities, which included occult dysrafism with sacral agenesia (N.2), lazy bladder (N.3) and vesico ureteral reflux (N. 3).

The extension of treatment was carried out through subsequent meetings that initially occur at 15 day intervals and are later reduced when the patient acquires adequate voiding behaviour and lack of symptoms.

Conclusions

Results show that medical attention, together with psychological counselling based in the cognitive techniques of behaviour recorded in the FVC, provide an effective, economical and non invasive approach to the treatment of children with recurrent UTI associated with LUTS.

With a simple behavioural approach most of the patients show a complete recovery from LUTS and recurrent UTI. Biofeedback pelvic floor muscle training is also effective in the treatment of dysfunctional voiding in children.[9,10] This conservative method of treatment has supplanted the common use of prophylactic antibiotics alone to manage recurrent UTI in children.[1] It is hoped however that paediatricians will adopt a more urodynamic "mentality", that makes use of this framework of signs and symptoms.

References

[1] McKenna PH, Herndon CD. Voiding dysfunction associated with incontinence, vesicoureteral reflux and recurrent urinary tract infections. Curr Opin Urol 2000 Nov; 10(6): 599-606

[2] Wan J, Kaplinsky R, Greenfield S. Toilet habits of children evaluated for urinary tract infection. J Urol 1995; 154: 797-799.

[3] O'Regan S, Yazbeck S, Schick E. Constipation, bladder instability, urinary tract infection syndrome. Clin Nephrol. 1985; 23(3): 152-154.

[4] Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-Committee of the International Continence Society. Neurol Urodyn, 2002; 21: 167-178.3

[5] Koff SA, Lapides J, Piazza DH. Association of urinary tract infection and reflux with uninhibited bladder contractions and voluntary sphincteric obstruction. J Urol 1979; 122(3): 373-376.

[6] Van Gool JD, De Jonge GA. Urge syndrome and urge incontinence. Arch Dis Child, 1989; 64(11): 1629-1634.

[7] De Gennaro M, Bergami F. Urodinamica clinica in urologia pediatrica. Cleup Editrice Padova. 1991.

[8] De Paepe H, Renson C, Van Laecke E, et al. Pelvic-floor therapy and toilet training in young children with dysfunctional voiding and constipation. BJU Int 2000; 85 (7): 889-93.

[9] Kuo HC, Liu HT. Investigation of dysfunctional voiding in children with urgency frequency syndrome and urinary incontinence. Urol Int. 2006; 76(1): 72-6.

[10] Schulman SL, Quinn CK, Plachter N et al. Comprehensive management of dysfunctional voiding. Pediatrics 1999; 103(3): E31.

Antimicrobial treatment for urinary tract infections

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Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections requiring antibiotic treatment. The major aims of treatment of UTIs are: relieve symptoms, prevent sepsis, relapse or recurrence of the

infection and avoid permanent renal damage. Recommendations for the management of UTI are conflicting and consistent differences persist among clinicians in treating affected children.

Treatment of acute urinary infections

Newborns and small infants, children with dehydration, toxicity, or sepsis are usually admitted to the hospital. For children who are vomiting or non-compliant with oral intake, the recommendation is to administer an antimicrobial parenterally. As an alternative to hospitalization Gauthier et al have proposed a day-care treatment [1].

Infants are commonly treated with ampicillin and gentamicin, which cover most of the common bacterial pathogens; in infants who need coverage for hospital associated infections vancomycin should substitute ampicillin. In older children a third-generation cephalosporin or an aminoglycoside are adviced. The initial antimicrobial choice should be adjusted after culture and susceptibility are known: in patients with compromised renal function, the use of potentially nephrotoxic antimicrobials (e.g. aminoglycosides) requires caution.

Acute pyelonephritis is commonly treated with parental antibiotics, but randomized controlled trials comparing oral vs parenteral treatment in febrile children with positive urine culture have shown no difference [2]. Agents that are excreted in the urine but do not achieve therapeutic concentrations in the blood-stream, such as nalidixic acid or nitrofurantoin, should not be used to treat UTI in febrile infants and young children in whom renal involvement is likely.

Uncomplicated cystitis in children can be treated with amoxicillin, amoxicillin-clavulanate (A/C), trimethoprim-sulfamethoxazole, nitrofurantoin or a first-generation cephalosporin, which reach adeguate concentrations in the lower urinary tract. The increasing use of third-generation cephalosporins has produced bacterial resistance to these drugs. Although the role of fluoroquinolones in pediatric UTI is still under investigation, the limited data available demonstrate a likelihood of efficacy and safety [3]; however a fluoroquinolone may be used in sexually mature teenagers and situations when no other alternative is reasonable.

Five retrospective observational studies found increased rates of renal scarring in children in whom diagnosis was delayed from four days. More recent studies [4] do not confirm this observation; it seems that a prolonged treatment will avoid the risk of renal damage.

Two meta-analysis have recently shown that the administration of the same total antibiotic dose by continuous intravenous infusion may be more efficient compared with the intermittent mode [5].

The recommended duration of antimicrobial therapy for uncomplicated cystitis is 7-10 days. Short-course therapy of cystitis is usually not recommended in children. Two apparently contrasting meta-analysis have been recently published [6-7]; from these studies it is clear that single dose or single day antibiotic treatment is not as effective as long-course treatment for UTIs in children, while "longer" short-course therapies may be as effective as 7-14 days of antibiotics.

Acute pyelonephritis is usually treated for 7-14 days; the duration of parenteral therapy in uncomplicated pyelonephritis is not well defined, but most children can complete therapy orally once symptomatic improvement has occurred. The inflammatory response to infection during an acute pyelonephritis in childhood may result in renal parenchymal scars which contribute to hypertension, renal disease, and renal failure later in life. Only a few studies on the long-term outcome of pyelonephritis and the effect of medical intervention on the development of adult renal diseases are available [4].

Prophylaxis

High risk for renal scarring has been reported in children with vesico-ureteric reflux, dilatation of the upper urinary tract and recurrent acute pyelonephritis; young age, bladder dysfunction, stones and urinary obstruction have also been associated with renal scars [8]. Indications for long-term prophylaxis include: infants with a febrile UTI; children up to 5 years with VUR grade 1-4; children with UTI and obstructive uropathy or frequent symptomatic recurrences. Two small randomized controlled trials (RCTs) found that prophylactic antibiotics may prevent recurrent urinary tract infections in children with VUR or recurrent UTIs; subsequently long-term antibacterial prophylaxis in infants and children started. The long term benefits of prophylaxis have not been adequately evaluated in children with vesicoureteric reflux [9-10]. Even more questionable is the indication of prophylactic antibiotics in infants with connatal dilatations of the upper urinary tract and neurogenic bladder dysfunction. Children with dysfunctional voiding generally do not benefit from prophylactic antimicrobials. Asymptomatic bacteriuria is detected in 0.5-1.0% of children who are screened with urine culture; treatment may increase the risk of symptomatic UTI by eliminating nonpathogenic colonization. Williams et al. believe that well-designed, randomized, placebo-controlled trials are still required to evaluate this commonly used intervention.

Antimicrobials selected for prophylaxis should: 1. be effective against most uropathogens, 2. cause few side effects, 3. produce minimal bacterial resistance, 4. respect the bacterial flora, 5. be inexpensive. Co-trimoxazole, trimethoprim, nitrofurantoin, sulfisoxazole are mostly used and effective drugs for prophylaxis. Use of broader spectrum antimicrobials may lead to colonization and infection with resistant strains, but amoxicillin-clavulanate and oral cephalosporins have been used as an alternative with success.

The optimal duration of long-term antibacterial prophylaxis in UTIs in children is unclear; the treatment should be continued until the risk of UTIs and renal scars is diminished.

Conclusions

UTI can be treated promptly and efficaciously in children nowadays. Antimicrobal resistance is increasing and should be considered when prescribing antibiotics for UTI. Despite many years of treating UTI we still do not know when an antibiotic should started, how long should be administered and if chemoprophylaxis is of any benefit in children with recurrent UTI or urinary tract malformations. Alternative treatments such as vaccines or bio-therapeutic agents may demonstrate similar efficacy with fewer side effects. Large, properly randomised, double blind trials are needed to solve these problems. Interesting data have recently been published on use of cranberry in pediatrics [11].

References

[1] Gauthier M, Chevalier I, Sterescu A, Bergeron S, Brunet S, Taddeo D. Treatment of urinary tract infections among febrile young children with daily intravenous antibiotic therapy at a day treatment center. Pediatrics. 2004; 114 (4): 469-76.

[2] Montini G, Toffolo A, Zucchetta P, Dall'Amico R, Gobber D, Calderan A, et al. Antibiotic treatment for pyelonephritis in children: multicentre randomised controlled non-inferiority trial. Br Med J. 2007; 335(7616):386.

[3] Koyle MA, Barqawi A, Wild J, Passamaneck M, Furness PD 3rd. Pediatric urinary tract infections: the role of fluoroquinolones. Pediatr Infect Dis J. 2003; 22 (12): 1133-7.

[4] Levtchenko E, Lahy C, Levy J, Ham H, Piepsz A. Treatment of children with acute pyelonephritis: a prospective randomized study. Pediatr Nephrol. 2001; 16 (11): 878-84.

[5] Kasiakou SK, Sermaides GJ, Michalopoulos A, Soteriades ES, Falagas ME. Continuous versus intermittent intravenous administration of antibiotics: a meta-analysis of randomised controlled trials. Lancet Infect Dis. 2005; 5 (9): 581-9.

[6] Keren R, Chan E. A meta-analysis of randomized, controlled trials comparing short-and long-course antibiotic therapy for urinary tract infections in children. Pediatrics 2002; 109: E70.

[7] Michael M, Hodson EM, Craig JC, et al .Short compared with standard duration of antibiotic treatment for urinary tract infection: a systematic review of randomised controlled trials. Arch Dis Child 2002; 87: 118-23.

[8] Beetz R. May we go on with antibacterial prophylaxis for urinary tract infections? Pediatr. Nephrol 2006; 21: 5-13.

[9] Garin EH, Olavarria F, Garcia Nieto V, Valenciano B, Campos A, Young L. Clinical significance of primary vesicoureteral reflux and urinary antibiotic prophylaxis after acute pyelonephritis: a multicenter, randomized, controlled study. Pediatrics. 2006;117(3):626-32.

[10] Fanos V, Cataldi L. Antibiotics or surgery for vesicoureteric reflux in children. Lancet 2004 ; 364 (9446): 1720-2.

[11] Fanos V, Atzei A, Zaffanello M, Piras A, Cataldi L. Cranberry and prevention of urinary tract infections in children. J Chemother 2006; 18 (congr rep n. 3): 21-24.