## LP2

### TABAGISME: NEW FRONTIERS OF LABORATORY MEDICINE IN PREVENTION AND DIAGNOSIS

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In Italy, likewise the whole occidental world, tobacco smoke remains the first avoidable cause of morbidity and mortality as known risk factor in respiratory, cardiovascular and neoplastic pathologies. Eighty-five thousands deaths per year are attributable to tobacco smoke, being the 25% in the range 35-65 years.
The World Health Organization declared that fight to tabagisme is the second important health priority, after the elimination of malaria.
In Italy, active smokers are around 12 millions, with a prevalence of 31.5 and 17.2 % in men and women, respectively (ISTAT 2000). In the recent years, percentage of active smokers between 14 and 24 years of age increased, as more that one fifty of young people smoke.
In the National Health Plan 2000-2004, the problem of tabagisme appears between strategic objectives, inside the promotion of healthy life styles, in the public prevention and communication of health.
Tabagisme is a pathologic condition, characterised by the need and habit to daily smoke cigarettes, which has to be diagnosed and treated.
Diagnosis of active or passive smoke can be complex and requires measurement of sensitive and specific biomarkers such as: quantification of COHb or CO in the expirate or detection of cotinine in different biological matrices (plasma, urine, saliva, hair).
On a clinical point of view, the acknowledgement of smoking habit in a patient is highly important during the therapy and in the evaluation of efficacy and compliance to a cessation treatment.
Therapy objective, which can be behavioural cognitive and/or pharmacological is to reach cessation. First choice medicaments are nicotine substitutives in the different pharmaceutical preparations (parches, inhalators, chewing-gums, sublingual tablets)and bupropion in the retard formulation. Relapses, as in all the chronic pathologies are frequent and the personalization of the intervention appears to be the most adequate strategy.
The different susceptibility of smokers to the tobacco-related damages, particularly lung and oropharyngeus cancer, are the subject of a great health interest.
Several studies show that the major cancer risk of tobacco smoke are due to the direct mutagenic action of "cancer-related" genes on DNA and the frequency of different types of mutations and the frequency of their appearance in those genes should be a risk factor objectively measurable.
The potential applicability of specific "carcinogen-derived" biomarkers is among the new challenges of laboratory medicine in the fight against tobacco-related pathologies.

## S3.1

### LABORATORY MEDICINE AND RENAL FAILURE: NEW PERSPECTIVES FOR THE EARLY ESTIMATION OF CHANGES IN GLOMERULAR FILTRATION RATE (GFR)

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Glomerular filtration rate (GFR) is widely believed to be the best overall index of renal function in health and disease and its accurate estimation is an important tool in the monitoring of renal function. Inulin and $^{51}$Cr Ethylenediaminetetraacetic acid ($^{51}$Cr-EDTA) clearances are traditionally considered "ideal markers" of GFR; however, they require specialized technical personnel over a period of several hours. In addition, a number of practical considerations (cumbersome methods of determination, radioactivity, high costs) have limited the use of these techniques in clinical practice. Serum creatinine and creatinine clearance are the most widely used methods for the routinary noninvasive estimation of GFR. Serum creatinine is considered relatively specific, but not very sensitive since its levels significantly increase when more than 50% of the GFR is reduced. Serum creatinine concentration may be significantly influenced by several extra-renal factors, such as muscle mass, changes in tubular secretion, dietary intake, analytical interferences, etc. Consequently, there is a need to provide an alternative to creatinine that may be analytically and clinically more reliable. Because the kidney plays a major role in the metabolism of low molecular mass (low-$M_r$) plasma proteins, it was postulated that serum levels of these proteins might reflect changes in GFR. Most of low-$M_r$ plasma proteins are freely filtered by the glomerulus and then almost completely reabsorbed and catabolized by proximal tubular cells. This means that they cannot return into the blood stream after their glomerular filtration. Until now, no protein has been definitively introduced in the clinical practice, because for most of them serum levels are significantly influenced by various extra-renal factors. Human cystatin C, alias γ-trace protein, is a basic low-$M_r$ plasma protein of 13,359 Dalton with two disulphide bridges, a positive charge at physiological pH, and an isoelectric point of 9.3. Containing one non-glycosylated polypeptyde chain with 120 amino acid residues, cystatin C belongs to a recently defined superfamily of proteins, called the cystatins superfamily, since all its members are cysteine proteinase inhibitors. The human cystatin C gene has been cloned, sequenced and located to chromosome 20p11.2, being of the so-called housekeeping type. Cystatin C is therefore steadily produced by all nucleated human cells and among cystatins, it is the one with the highest molar concentration in seminal plasma, in the milk, and in the cerebrospinal fluid, where it is actively secreted by the choroid plexus. Its production is not influenced by inflammation and thus it cannot be considered an acute phase protein. Cystatin C does not cross the placental barrier. The serum cystatin C concentration seems to be not significantly influenced by gender and age beyond the first year of life. It was demonstrated that the renal clearance of cystatin C is closely related to the GFR, measured as $^{51}$Cr-EDTA clearance. Cystatin C increases considerably in patients with renal failure. Over the last five years, a moltitude of clinical studies on patients with various renal diseases investigated the diagnostic accuracy of cystatin C, comparing its sensitivity ans specificity with those of traditional indexes (serum creatinine, creatinine clearance, Cockcroft and Gault estimated GFR, etc). Most of the studies found that cystatin C diagnostic accuracy is better than that of serum creatinine in discriminating patients with reduced GFR from those with normal GFR. In particular, one of the most significant advantages of cystatin C in clinical nephrology seems to be that very small reductions in GFR cause significant increases in cystatin C serum levels. It was also postulated that cystatin C and creatinine are two independent markers of GFR, because several studies have found that they significantly correlate each other in patients with reduced GFR, but not in subjects with normal GFR. The routinary measurement of cystatin C seems to be of particular value in neonatology, in the monitoring of renal transplant patients, in the course of diabetic nephropathy, in the early assessment of fetal uropathies, and in all the clinical conditions characterized by a small decrease in GFR without any concomitantly change either in serum creatinine levels or in creatinine clearance values.

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## S3.2

### PROTEINURIA: A USEFUL AID IN DEFINING GLOMERULAR WALL INJURY

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Proteinuria is characterized by an abnormal amount (more than 150 mg/day) of proteins in the urine of a healthy adult male subject. It is defined as "glomerular" when proteins with a molecular weight superior of 65,000 Dalton are mainly present, as "tubular" when proteins have usually a molecular weight between 10,000-25,000 Dalton are more represented or "mixed" when both types of proteins are present. While tubular proteinuria signifies the inability of the renal tubule to reabsorb the low molecular weight component of the filtered glomeular load, glomerular proteinuria is a sign of an injury in the glomerular filtering barrier.

The glomerular filtering barrier, opposing the transit of proteins, is composed of at least 3 layers: the fenestrated endothelial wall that just hinders the passage of high molecular weight proteins; the glomerular basement membrane composed of a thick net of collagen IV, laminin, eparansulphate-rich (agrine) proteoglycans that opposes the transit both by steric and electrostatic hindering, the last due to an elevated electronegativity. However the more selective structure for the transit of proteins is the zone between opposing foot processes of the podocytes, called 'slit diaphragm'. This diaphragm is a zipper–like structure, sticklet-like molecules connecting at right angle with a long linear molecule between the foot processes. Molecular biology allowed now to know some of these proteins: nephrin, CD2AP and podocyn.

An early sign of a damage in the glomerular filtering barrier is the appearance of microalbuminuria (MA), which means an abnormal urinary excretion rate of albumin, in the range between 30 and 300 mg per day.

Microalbuminuria (MA) is present in 5-40 % of the subjects with essential hypertension and in 25 % of those with diabetes mellitus type 1. In the first population there is a correlation between the appearance of MA and the "non-dipper" and "non-modulating" subset; in the other population there is a correlation with the duration of diabetes, inadequate metabolic control of the disease and the presence of hypertension.

The persistence of an immunologic injury and the worsening of the intraglomerular hemodynamic due to a the reduction of the nephron mass cause a worsening of proteinuria from MA to a dosable proteinuria range with a glomerular pattern. The augmented filtered load of proteins determines phenotipic alterations on the proximal tubular cells and through activation of NF-kB and further production of chemokines (RANTES, MCP1, INF, etc), it causes tubular injury and the activation of a cell-mediated reaction involving interstitium, which will eventually cause chronic interstitial fibrosis and kidney shrinkage.

In these conditions, the initial glomerular proteinuria will develop an additional tubular component and a mixed glomerulo-tubular pattern will ensue. Elegant researches by Bazzi C. and coll. studying proteinuria patterns on SDS-PAGE layers successively compared with the renal function and histology, have confirmed the tight correlation between the degree of renal failure, severity of tubular interstitial damage and the amount of tubular component.

The accumulated experience in the last 15 years has shown the efficacy of ACE inhibitors and, more recently, of receptor antagonists of angiotensin in the reduction of proteinuria and hence in the slowing of renal failure progression through the removal of the hemodynamic alterations induced at glomerular level by angiotensin II.

In addition, very recent studies seem to show that these drugs interact in the composition of the slit diaphragm and hence with podocyte function as shown by the ability of ACE inhibitors in preventing the down-regulation of nephrin gene, usually noted in Heyman's nephritis.

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## S3.3

### EVALUATION OF RENAL TUBULAR FUNCTION: FROM THE LABORATORY TO CLINICAL DIAGNOSIS

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A main function of the renal tubule consists in the regulation of body fluid volume and composition: this is achieved by selective reabsorptive/secretory processes on the glomerular ultrafiltrate (about 180 L/day) and its components: $H_2O$, electrolytes, organic anions/cations, non-volatile determinants of acid-base homeostasis, etc. Abnormal tubular function may thus result in abnormal plasma levels of specific components (e.g. hypo-hyperkalemia, ipo-hypermagnesemia, acidosis or alkalosis, etc); moreover, changes in plasma solute levels secondary to extrarenal events are associated with compensatory responses of the renal tubule, which may be appreciated through urine evaluation. Thus, contemporary evaluation of plasma and urine solute levels may be of much help in the evaluation of many disorders characterized by abnormal plasma levels/urine excretion of solutes, specifically electrolytes and determinants of acid-base homeostasis.

Generally speaking, renal regulation of plasma solutes occurs around a "threshold" level in a context of a positive ongoing load of solutes into the blood; this mechanism allows for a quantitative excretion into the urine of all the fraction of filtered load of a solute exceeding its "plasma threshold"; if net load of any solute into the blood ceases (or becomes negative, as it may occur with extrarenal fluid losses), the filtered load becomes less than plasma threshold and urine excretion falls to minimal levels (approaching to zero for some solute, e.g. Na, Mg, Cl). In this context the kidney becomes unable to regulate plasma levels.

When tubular function is impaired, urine solute excretion may not fall (or rise) with the reduction/increase of plasma levels, indicating that plasma threshold is lower or higher than normal. This occurs in many tubular disorders associated with hypo-hyperkalemia, hypo-hypermagnesiemia, hypophosphatemia, metabolic acidosis/alcalosis, etc. Evaluation of urine solute levels in parallel with plasma levels may thus allow to differentiate renal from extrarenal causes of abnormal plasma solute regulation.

For tubular Na handling, plasma regulation occurs around extracellular fluid volume status rather than plasma Na concentration itself; thus, urine Na excretion is a useful parameter of extracellular fluid volume (EFV), low levels (usually < 10-15 mM) indicating contraction of effective EFV. For several solutes (such a glucose, amminoacids and other organic compounds) plasma levels are mostly dependent on cellular metabolic events, so that abnormal tubular handling is attended by increased urine excretion without any plasma level reduction.

Since different tubular segments express functional specialisation, tubular disorders may involve groups of solutes; e.g. proximal tubular disfunction is associated with urine loss of glucose, aminoacids, $HCO_3^-$, phosphate, urate, ensuing in hypophosphatemia, hypouricemia, metabolic acidosis, normoglycemic glucosuria and aminoaciduria, the so called Fanconi syndrome. In distal nephron disorders abnormal handling of Na/Cl, Ca, Mg, K may result in hypokalemia, hypercalciuria/nephrocalcinosis, hypovolemic hypotension, as shown in Barrter syndrome; collecting tubule disorders are characterized by abnormal handling of $H_2O$, K, $H^+/HCO_3^-$, resulting in polyuria, hyperkalemia, metabolic acidosis, as exemplified in nephrogenic diabetes insipidus, pseudohypoaldosteronism type 1 and 2, and distal renal tubular acidosis, respectively.

Urine solute excretion may be evaluated by several ways: urine solute concentration is a sinple method which may be of help for electrolytes and urine acidification parameters; the urine solute to creatinine concentration ratio (Ux/Ucr) allows for a semiquantitative evaluation of solute excretion, such as for Ca, Mg, uric acid. Quantitative excretion (quantity/day or quantity/min) is most useful for balance studies and dietary evaluation; factorialisation for GFR (quantity/ dlGFR; Clx/GFR, which equals quantity/ filtered load, usually indicated as FEx) is usually taken as a direct evaluation of tubular reabsorptive/secretory capacity, and is frequently performed in acute functional studies. If creatinine is used as a GFR index, quantity/GFR and FEx can simply be calculated as (Ux/Ucr * Pcr) and (Ux/Px * Pcr/Ucr), respectively, which do not require urine volume and collection time.

It has to be stressed that urine solute excretion has to be evaluated in parallel with plasma levels to allow the best appreciation of tubular function; daily urine solute excretion, in the steady state, represent more dietary intake than tubular handling.

It is possible to evaluate quantitative tubular reabsorptive/ secretory capacity "in vivo" by performing dynamic tests, whereby by an exogenous load (e.g. $NH_4Cl$, phosphate, $HCO_3^-$, $H_2O$) is given; these dynamic tests usually allow definitive diagnostic informations on renal tubular function.

## S3.4

# ACQUIRED ANEMIA: THE DIAGNOSTIC CHALLENGE

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In the multiethnic society the role of the haematological laboratory is growing and it is crucial to distinguish acquired anemias from chronic diseases, typical of Western countries, and anemias from parasitical infestation or dietary scantiness, typical of immigrants from developping countries. The diagnosis of acquired anemias is, actually, characterized by mixing of quoted factors; for example, parasitical diseases or problems owing to incorrect or insufficient diet could be found in Western people who travel in exotic countries or follow improper lifestyles.

The automation and robotization of haematology deeply changed the organization of laboratory and the diagnostic role of pathologist. The high throughput of automated systems allowed the use of haematological paramaters in diagnosis and follow up in wide clinical and preventive medicine fields; the availability of new parameters of erythrocytes and subpopulations of leukocytes allowed a rising accuracy of diagnostic data; the improvement of informatics permitted univocal and positive identification of tubes and interpretation of cells and particles distribution on the basis of size and of peculiar treatment with solutions and dyes; the robotization of analysis limited the human control and manpower and a continuous utilization of the instruments with an improvement of turnaround time and of timely supply of data to the clinicians. The laboratory haematologist had the possibility to interpretate high amount of data and to propose to the clinicians new concepts about the parameters modifications in diseases, in association with the classical microscopical examination of blood smears. The neglect link between laboratory and clinical haematologist and the spreading of instruments in laboratories, haematology wards, blood banks, however, did not completely develop the opportunities supplied by automation.

The diagnosis of anemia could represent a paradigm of the need of link between automation and related parameters on erythrocytes and reticulocytes and laboratory evaluation based on clinical data and microscopy, in association with other clinical chemistry analytes for studying iron and folates metabolism.

The acquired anemias in Western countries are clearly linked to ageing of population and the growing of chronic diseases. The anemia caused by chemotherapy and (or) malignancies is more and more frequent, and the use of erythrocytes parameters and reticulocytes enumeration , volume, and haemoglobin content are useful to detect the anemia source and to describe characteristics of particles (anisopoichilocytosis and echinocytosis, with haemolysis). The chronic diseases as rheumatoid arthritis and chronic liver diseases can induce a sideropenic (or megaloblastic or mixed forms) anemia, but it is interesting to remark that some infectious diseases as AIDS and the "reviving" tubercolosis are now chronic conditions and are inevitably accompanied by sideropenic anemia and by some particular characteristics of cells distribution (lymphocytes) due to different ion cellular membrane transport. The dialysis allowed chronic renal impaired patients to a quite regular lifespan, but a dangerous anemia is always present in these patients, partially solved by the use of erythropoietin and novel erythropoietins which can induce, however, neutralizing antibodies. In this case, the automated haematology permits an accurate followup of anemic haemodialyzed with reticulocytes number, volume and haemoglobin content.

The modification of lifestyle of part of Western people induced some improvements of diets, proposing a reduction of hyperchaloric ones, and a stimulus for physical exercise and fitness. This behaviour could induce, however, exaggerated and compulsory paraphysiological or clearly pathological conditions, characterized by sideropenic anemia and hypovitaminosis, which could be suspected from haematological parameters, especially erythrocytes volume and haemoglobin content. In amateur and elite athletes, especially females, the "sport's anemia" should be recognized and treated; the haematological monitoring is crucial to avoid iron metabolism problems and pitfalls. The condition of "athletic triad" of women runners with the paradox of amenorrhoea, iron deficiency and osteoporosis could describe the "new" syndromes that the pathologist can meet. At the contrary, the clinical laboratory is now claimed to study and follow the haemodoping, particularly with erythropoietin and similar bone marrow stimulants, by using erythrocytes parameters and reticulocytes ones. The use of haemodoping is sometimes mimicking the anemia treatment and the diagnostic challenge is evident.

The immigration from Asia, Africa, East Europe and Latin America of a numerous people strenghtens the need to suspect and diagnose anemias caused by haemoglobinopathies and parasitic infections. In these cases, the automated instruments can point out some modifications of erythrocytes parameters, interference in leukocytes counts by parasited erythrocytes, and modifications of leukocytes plots due to residual parasitic pygments in neutrophils, but the microscopical examination is fundamental to diagnose malaria or babesios and mandatory for extracellular blood parasites. It is evident that the quality control for haematology laboratory should not be only directed to accuracy and precision of the instruments but should be directed also to these diagnostic problems. In this case, the e-learning and the distribution via Internet of blood smears are an important tool for improving the general knowledge, as demonstarted by Italbioforma project.

The diagnosis of anemia or the suspect of preanemic conditions is now supported by the availability of various reticulocyets parameters (count, volume, haemoglobin content, immature distribution). The accuracy, precision, and stability of these parameters are sufficient to the routine purposes, but the interlaboratory variability among systems, owing to the use of different dyes and technologies for particle counting, and difficult evaluation of critical difference, due to intra- and interindividual variability, should limit the clinical usefulness of reticulocytes parameters.

The diagnosis of anemia should be now released also out of laboratory by using novel instruments of point of care testing. The control and validation of these systems must be determined by laboratory professionals, who should be aware about the importance of point of care testing spreading.

## S3.5

# RED CELL MEMBRANE AND ENZYME DEFECTS ASSOCIATED WITH HEMOLYTIC ANEMIA

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Inherited defects of red cell membrane and metabolism are the most common causes of congenital hemolytic anemia.

*Enzyme deficiencies* have been identified in all erythrocyte metabolic pathways. Abnormalities of the hexose monophosphate shunt (glucose 6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase, and enzymes of glutathione synthesis) are more often characterized by acute hemolytic anemia induced by oxidative stress. Defects of glycolysis and nucleotide pathways result in chronic hemolytic anemia alone (pyruvate kinase, glucose phosphate isomerase, hexokinase, pyrimidine 5' nucleotidase, adenylate kinase), or associated with more complex symptoms if the affected enzyme is not confined to red cells but is also expressed in other tissues (aldolase, phosphoglycero kinase, phosphofructo kinase, triosephosphate isomerase). The diagnosis of red cells enzyme deficiency is based on the exclusion of the most common causes of haemolytic anaemia and on the demonstration of a reduced enzyme activity. Care must be taken in interpreting in vitro enzyme assays: contamination with normal donor cells in recently transfused patients, incomplete leukocyte removal or high reticulocyte number may cause false negative results. The evaluation of kinetic parameters and enzyme stability may be helpful in doubtful cases. Over the past few years the inherited disorder of red cell metabolism have been the object of intensive research which has resulted in a better understanding of their molecular basis (1-3 ). Numerous mutations responsible for the most common enzyme defects have been identified, allowing prenatal diagnosis and genetic counselling. As regards pyruvate kinase (PK) deficiency, which is the most common glycolytic enzyme abnormality, more than 130 different mutations have been so far reported (4). Clinical and molecular studies on 31 consecutive PK deficient patients (Table 1) revealed that mild clinical signs were associated with compound heterozygosity for two missense mutations (with a prevalence of 1456T, the most frequent mutation in Caucasians), or for one missense and a more drastic mutation involving terminal exons of the gene. Severe clinical expression was associated with a) disruptive mutations; b) missense mutations involving the Aa7 helix, even in association with a mild mutation as 1456T; c) mutations 994A and 1529A at the homozygous state (5).

*Red cell membrane defects* comprise hereditary spherocytosis, elliptocytosis and stomatocytosis. Hereditary spherocytosis is the most common hemolytic disorder in Caucasians, its prevalence ranging from 1:2000 to 1:5000. The disease is highly heterogeneous at both clinical, biochemical and genetic level (6-7). Splenectomy is beneficial in most severe cases (8). The disease is caused by a defect of the red blood cell membrane proteins, either peripheral or integral ones: spectrin, ankyrin, band 3 and band 4.2. The diagnosis is based on the presence of spherocytes and microspherocytes in the peripheral blood smear, reticulocytosis, and increased red cell osmotic fragility. SDS-PAGE analysis of red blood cell membrane proteins allows the identification of the molecular defect in most patients (Table 2) (9). Hereditary elliptocytosis is a more rare disease and diagnosis is based on the presence of elliptocytes in peripheral blood. The underlying defects are deficiency of protein 4.1 or qualitative abnormalities of spectrin. The identification of molecular abnormalities requires SDS-PAGE analysis, functional studies and tryptic digestion of spectrin (Table 3).

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Tab. 2
SDS-PAGE analysis of 160 patients affected by Hereditary Spherocytosis

| Defect | Total cases | | Not splenectomized | | Splenectomized | |
|---|---|---|---|---|---|---|
| | N. | % | N. | % | N. | % |
| Band 3 | 52 | 33 | 42 | 31 | 10 | 38 |
| Spectrin | 44 | 27 | 34 | 25 | 10 | 38 |
| Band 4.2 (*) | 13 | 8 | 13 | 10 | 0 | 0 |
| Ankirin (**) | 13 | 8 | 9 | 7 | 4 | 16 |
| Unidentified | 38 | 24 | 36 | 27 | 2 | 8 |
| Total | 160 | 100 | 134 | 100 | 26 | 100 |

(*) secondary to a band 3 defect
(**) including 6 ankyrin-deficient patients and 7 ankyrin and spectrin-deficient patients

Tab. 3
SDS-PAGE analysis and/or spectrin functional study of 21 patients affected by Hereditary Elliptocytosis

| Defect | Total cases | |
|---|---|---|
| | Nr | % |
| Spectrin variant al74 | 4 | 19 |
| Spectrin variant al65 | 4 | 19 |
| Spectrin variant al46 | 3 | 14 |
| Spectrin variant V41 | 2 | 10 |
| Band 4.1 deficiency | 7 | 33 |
| Unidentified | 1 | 5 |
| Total | 21 | 100 |

Table 1. Clinical , hematological and molecular data of the PK deficient patients studied.

| Case | Sex | Age at Diagnosis (yr) | Neonat Jaundice | ExTx | Tx (units) | Spleen | Hb (g/dL) | Retics ($10^9$/L) | Unconj. Bilirubin (mg/dL) | Serum ferritin (µg/L) | PK Activity (IU/gHb) | Mutation | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **MILD PHENOTYPE** | | | | | | | | | | | | |
| 1 | M | 16 | Yes | Yes | 3 | In | 10,4 | 166 | 5,46 | 88 | 4,5 | 1456T/1456T | Arg486-Trp/Arg486-Trp |
| 2 | F | 30 | No | No | 0 | In | 11,6 | 167 | 1,27 | 140 | 4,2 | 1456T/1552A | Arg486-Trp/Arg518-Ser |
| 3 | M | 31 | No | No | 0 | In | 12,5 | 123 | 3,57 | 76 | 3,6 | 1456T/1675T | Arg486-Trp/559Arg-End |
| 4 | F | 19 | No | No | 0 | In | 10 | 303 | 0,45 | 94 | 2,8 | 1456T/ ? | Arg486-Trp/? |
| 5 | F | 2 | Yes | Yes | 0 | In | 11,2 | 169 | 1,03 | 116 | 3,0 | 1456T/823A | Arg486-Trp/Gly275Arg |
| 6 | F | 43 | No | No | 0 | In | 10,8 | 198 | 1,75 | 186 | 5,8 | 1456T/993A | Arg486-Trp/331Asp-Glu |
| 7 | M | 18 | No | No | 2 | In | 11,6 | 222 | 2,4 | 810 | 3,1 | 514C/514C | Glu172-Gln/Glu172-Gln |
| 8 | F | 24 | No | No | 0 | In | 10,6 | 190 | 2,03 | 23 | 2,9 | 1594T/ 993A | Arg532-Trp/331Asp-Glu |
| 9 | F | 24 | No | No | 0 | In | 12,9 | 130 | 3,97 | 186 | 4,0 | 1168A/? | Asp390-Asn/? |
| 10 | F | 21 | No | Yes | 0 | In | 10,5 | 149 | 6,81 | 214 | 9,0 | 1456T/1151T | Arg486-Trp/384Thr-Met |
| 11 | M | 35 | Yes | No | 0 | In | 15,6 | 125 | 2,4 | 153 | 7,2 | 1456T/? | Arg486-Trp/? |
| 12 | F | 27 | Yes | Yes | 1 | In | 9,2 | 218 | 3,5 | 190 | 2,4 | 1456T/del 1042-1044 | Arg486-Trp/del 348Lys |
| 13 | M | 13 | No | No | 0 | In | 11,2 | 88 | 0,5 | 75 | 2,6 | 1456T/del 1515-1518 | Arg486-Trp/frameshift |
| 14 | F | 36 | Yes | No | 4 | In | 11,6 | 81 | 0,4 | 484 | 2,8 | 991A/? | Asp331/Asn |
| 15 | F | 8 | Yes | No | Yes | In | 10.3 | 146 | nd | nd | 5.9 | 1456T/1232G | Arg486Trp/Gly411Ala |
| 16 | M | 16 | No | No | 0 | In | 12.4 | 53 | nd | nd | 3.2 | 721T/1456T | Glu241End/ Arg486Trp |
| | **SEVERE PHENOTYPE** | | | | | | | | | | | | |
| 17 | F | 40 | No | No | 0 | Out | 9,5 | 1452 | 0,44 | 540 | 1,3 | 1529A/1529A | Arg510-Gln/Arg510-Gln |
| 18 | M | 12 | Yes | Yes | >50 | Out | 8,5 | 263 | 1,59 | 790 | 9,7 | 1529A/994A | Arg510-Gln/Gly332-Ser |
| 19 | M | 6 | Yes | Yes | >50 | Out | 7,4 | 1046 | 1,97 | 2800 | 3,5 | 994A/994A | Gly332-Ser/Gly332-Ser |
| 20 | M | 21 | No | Yes | >100 | Out | 7,4 | 1252 | 5,62 | 716 | 5,5 | IVS3(-2)c/721T | splice site/Glu241-End |
| 21 | F | 6 | Yes | Yes | >100 | Out | 8,3 | 113 | 1,56 | 436 | 2,8 | 1269C/ 787A | splice site/263Gly-Arg |
| 22 | F | 6 | Yes | No | >50 | In | 6,9 | 154 | 3,3 | 1440 | 8,3 | Del 227-231/? | Frameshift/? |
| 23 | M | 19 | Yes | No | >100 | Out | 8 | 1124 | 15,0 | 1174 | 2,6 | 1456T/1160G | Arg486-Trp/387Glu-Gly |
| 24 | F | 6 | Yes | Yes | 1 | Out | 7,8 | 360 | 1,79 | 433 | 4,8 | 1456T/1181T | Arg486-Trp/394Ala-Val |
| 25 | M | 24 | Yes | Yes | >100 | Out | 8,9 | 949 | 5,1 | 612 | 13,0 | 1483A/ Ivs6 (-2)t | Ala 465-Thr/ del es10 |
| 26 | M | 9m | Yes | No | 7 | In | 6,8 | 149 | Nd | Nd | 15,6 | 1456T/1181A | Arg486-Trp/394Ala-Asp |
| 27 | M | 3m | Yes | No | >50 | Out | 6,1 | 249 | 0,11 | 828 | 9,5 | 721T/507A | Glu241-End/Splice site |
| 28 | F | 1 | Yes | Yes | 6 | In | 5,3 | 116 | nd | nd | 8,2 | 278T/1456T | Thr93Ile/Arg486Trp |
| 29 | F | 1 | Yes | Yes | 10 | In | 8.4 | 134 | nd | nd | 5,4 | Del 1437-1618 t | Frameshift/Frameshif |
| 30 | F | 11 | Yes | Yes | >50 | Out | 8.2 | 301 | nd | nd | 3,6 | 1484T/ IVS9 –1t | Ala495Val/Splice site |
| 31 | F | 6m | Yes | Yes | 4 | In | 6.8 | 322 | nd | nd | 12.1 | 994A/994A | Gly332Ser/Gly332Ser |
| Reference values | | | | | | | 12,2-16,7 | 24-84 | <0,75 | 15-355 | 11.1-15,5 | | |

Tx= transfusions; ExTx= Exchange transfusion; nd= not determined

## S3.6

HEMOGLOBINOPHATIES AND THALASSEMIAS

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The thalassemias syndromes and the hemoglobinophaties are widespread throughout the Mediterranean region, Africa, the middle East, the Indian subcontinent, Southeast Asia including China, the Malay peninsula and Indonesia. The population of Northern Europe and the Euro-Mediterranean area is bound to grow into a new society with a strong multi-ethnic character. In this process interaction between the different countries is becoming more and more essential for the public health of our new society. In this the confrontation with the problem of equal access to health information, diagnosis, health care and prevention becomes inevitable. On the field of severe inherited diseases such as thalassemia major and sickle cell disease, the experience acquired in several Mediterranean and North-European countries (Greece, Italy, Malta, Cyprus, England, France, The Netherlands etc...) has shown that, whenever offered, primary prevention is the alternative of choice of most couples at risk in well-informed autochthonous and allochthonous populations. This because of the severe pathology, the intensive supportive therapy and the emotional, financial and social burden associated with a child with thalassemia major or hemoglobinophaties. In order to improve the level of healthcare, patients must be studied and new therapeutical approaches must be designed and tested. For primary prevention couples at risk must be identified. The last using suitable elements of information and carrier diagnostics or screening. Advanced technical tools and molecular background of the population at risk must be tested or/and studied, developed, optimalized or just provided where absent. The choice for an appropriate medical approach adapted not only to the scientific evidences but also to the social and cultural and economical situation will have dramatic consequences for the utilization, the acceptance or the refusal of care. Similarely information adapted to a multiethnic society will give acces to counseling and primary prevention without fear of stigmatization or shame. Training of diagnostic and therpeutical experts as well as genetic counselors and nurses will add great value to the healthcare situation of a number of neglected countries and to the situation of immigrant in Northern-Europe. Sharing knowledge and experiences among countries and approaching health care problems in emerging countries and in the ethnic minorities of the immigration areas of Northern-Europe will contribute to equal access to healthcare and to a healthier society. This will imply the development of the new lines of health care in the multi-ethnic society of this newborn century.