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Editorial

The recent Emma's Children Hospital scientific meeting 2006 has been a great success with excellent presentations in many different pediatric disciplines. In this special issue of the Pediatric Clinics Amsterdam you will find the papers of the young investigators who were selected to present their research data in a master class.

Koert M. Dolman, editor in chief

Cardioprotective interventions for cancer patients receiving anthracyclines: a Cochrane systematic review

E.C. van Dalen¹, H.N. Caron¹, H.O. Dickinson², L.C.M. Kremer¹

Introduction

Anthracyclines have gained widespread use in the treatment of numerous malignancies in both adults and children. Unfortunately, their use is limited by the occurrence of cardiac damage. The precise mechanism underlying this is still not fully understood, but a major role has been ascribed to free radical generation by anthracycline-iron complexes. The heart is particularly vulnerable to free radical injury because protective enzymes such as superoxide dismutase are present at a lower level in

heart tissue than in other tissues.¹ The cardiac damage can become manifest as either clinical heart failure or asymptomatic cardiac dysfunction, which may not only develop during anthracycline therapy, but also years after the cessation of treatment.² The risk of developing heart failure therefore remains a lifelong threat, especially to children who have a long life-expectancy after a successful antineoplastic treatment. In the literature the frequency of clinical heart failure has been reported to be as high as 16%³ and that of asymptomatic cardiac dysfunction to be more than 57%.⁴ Several risk factors, such as a higher cumulative anthracycline dose, cardiac radiotherapy and female gender, have been identified, although not conclusively in all studies.^{3,4} In an effort to prevent or reduce cardiac damage,

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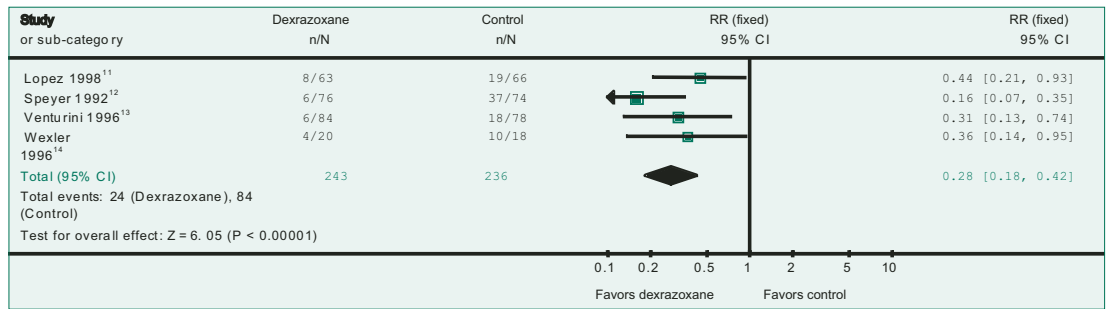


Figure 1. Meta-analysis of clinical heart failure and asymptomatic cardiac dysfunction combined in cancer patients treated with dexrazoxane versus control treatment.

N: total number of patients in group; n: number of patients with the event; RR: relative risk; CI: confidence interval

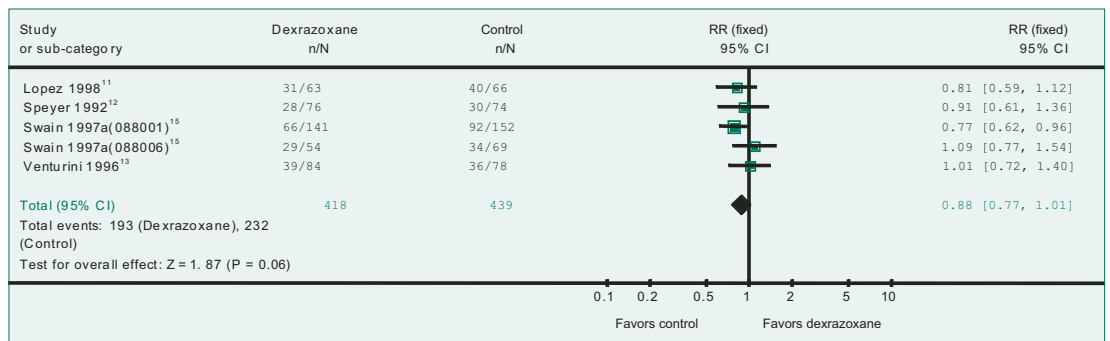


Figure 2. Meta-analysis of tumor response in cancer patients treated with dexrazoxane versus control treatment

N: total number of patients in group; n: number of patients with the event; RR: relative risk; CI: confidence interval

extensive research has been devoted to the identification of cardioprotective agents. Dexrazoxane is the most generally investigated agent⁵, but cardioprotective effects of other agents such as L-carnitine⁶ and coenzyme Q10⁷ have been reported.

At present, no systematic review on cardioprotective interventions during anthracycline therapy has been carried out. The objective of this review was to assess the efficacy of different cardioprotective agents in preventing cardiac damage in cancer patients treated with anthracyclines and to determine possible effects of these cardioprotective interventions on tumor response, survival and toxicities other than cardiac damage.

Methods

To be included in this review a study had to be a randomized controlled trial (RCT) investigating a cardioprotective intervention in children and/or adults with cancer treated with anthracyclines as compared to placebo or no additional treatment. We performed an extensive literature search. Appropriate search terms for the different cardioprotective agents (dexrazoxane, L-carnitine, probucol, coenzyme Q10, N-acetylcysteine, vitamin E, digoxin, ACE inhibitors, phenethylamines, deferoxamine, EDTA, guanidines, cytochromes, vitamin C, superoxide dismutase and monohydroxyethylrutoside), anthracyclines and RCTs were combined. Our search not only included the databases of Medline, EMBASE and CENTRAL of the Cochrane Library (up to August 2002), but also the conference proceedings of ASCO (American Society of Clinical Oncology) and SIOP (International Society for Pediatric Oncology) (from 1998 to 2002) and reference lists of relevant articles. Furthermore, we asked experts in the field if they were aware of any unpublished or ongoing studies. No language restriction was imposed.

Identification of studies meeting the inclusion criteria and data extraction was undertaken by two reviewers

independently. To identify possible bias, the quality of the included trials was assessed according to the following criteria: concealment of treatment allocation (selection bias), blinding of care providers and patients (performance bias), blinding of outcome assessors (detection bias), and completeness of follow-up (attrition bias). The data were analyzed in RevMan software according to the guidelines of the Cochrane Handbook⁸. A meta-analysis was performed for each cardioprotective intervention for which three or more studies were identified.

Results

We identified RCTs for 5 cardioprotective agents: N-acetylcysteine (1 study; 54 adults), phenethylamines (2 studies; 100 adults), coenzyme Q10 (1 study; 20 children), combination of vitamin E, vitamin C and N-acetylcysteine (1 study; 14 adults) and dexrazoxane (6 studies; 1013 patients, mostly adults). All studies had methodological limitations.

Due to the insufficient number of studies, pooling of the results was impossible for the first four mentioned cardioprotective agents. However, none of the individual studies showed a cardioprotective effect.

The meta-analysis of the dexrazoxane studies showed a statistically significant benefit in favor of dexrazoxane for the occurrence of both clinical heart failure (RR=0.18, 95% CI 0.10 to 0.35, P < 0.00001) and clinical heart failure and asymptomatic cardiac dysfunction combined (RR=0.28, 95% CI 0.18 to 0.42, P<0.00001; see Figure 1). No statistically significant difference in response rate between the dexrazoxane and control group was found (RR=0.88, 95% CI 0.77 to 1.01, P=0.06; see Figure 2), but there was some evidence that patients treated with dexrazoxane might have a lower response rate. Our meta-analysis of survival showed no significant difference between the dexrazoxane and control group (progression-free survival: HR=1.13, 95% CI 0.95 to 1.35, P=0.18 and overall survival: HR=1.07, 95% CI 0.89 to 1.28,

$P=0.49$). Pooling was impossible for adverse effects. However, no important differences in the occurrence of side effects were found. The majority of the patients included in this meta-analysis were adults with advanced breast cancer.

Discussion

For dexrazoxane, our meta-analysis clearly showed its efficacy in preventing cardiac damage in patients treated with anthracyclines. Despite this positive result, dexrazoxane is not routinely used because of potential side effects, such as possible interference with anti-tumor efficacy. We did not find a statistically significant difference in response rate between the dexrazoxane and control group in our meta-analysis, but there was some evidence that patients treated with dexrazoxane might have a lower response rate. However, the methods used for determining the response rate are likely to have a wide observer variance. Only 2 studies mentioned that the response rate was determined by at least two observers, thereby limiting the risk of unreliable results. It should also be mentioned that response rate is a surrogate marker for survival and the predictive value of response rate for survival is not clear⁹. In our meta-analysis of both progression-free and overall survival no significant difference between the dexrazoxane and control group was found. Also, although this systematic review does not allow for a definitive conclusion, no important differences in the occurrence of side effects were found in the included studies. We conclude that if the risk of cardiac damage is expected to be high, it might be justified to use dexrazoxane in patients with cancer treated with anthracyclines. However, for each individual patient clinicians should weigh the cardioprotective effect of dexrazoxane against the possible risk of a lower response rate.

Future trials on dexrazoxane should focus primarily on survival, response rate and adverse effects. They should be performed in homogeneous study populations treated for either a hematological malignancy or a solid tumor. Also, since data obtained in adults cannot be extrapolated to children, dexrazoxane should be evaluated in children. At the moment, we are aware of three ongoing trials in children. We are awaiting the results of those trials.

For interventions for which no RCTs were identified and for interventions for which pooling of results was not possible, no (definitive) conclusions can be made about the efficacy of these interventions in preventing cardiac damage in patients treated with anthracyclines. High quality RCTs with adequate power need to be undertaken.

Footnote

This paper is based on a Cochrane review, which is available in The Cochrane Library.¹⁰ Cochrane systematic reviews are regularly updated to include new research, and in response to feedback from readers. The Cochrane Library should be consulted for the most recent version of the review.

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Medullary thyroid carcinoma in children

J. Dols, H.M. van Santen, D.C. Aronson, C. vd Bos, W.M. Wiersinga and T. Vulsma

Introduction

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor, which is derived from the parafollicular cells, or C-cells, of the thyroid gland. MTC may be sporadic (75% of the cases in adults) or may occur as a manifestation of the hereditary syndrome Multiple Endocrine Neoplasia type 2 (MEN2). MEN2 includes tumors of thyroid C-cells and adrenal medulla and

hyperplasia or adenoma of the parathyroids. The clinical subtypes of MEN2, MEN2A and MEN2B, are defined by the combination of tissues affected and the presence or absence of developmental abnormalities. The incidence of MEN2A and MEN2B is about 2 - 3 per 100,000. The familial forms are autosomal dominantly inherited.¹

The thyroid gland is always involved in MEN2 patients; nearly 100% of the patients develop MTC. Patients with MEN2A and MEN2B also have an increased risk of pheochromocytoma.²

MEN2A shows no distinct physical features. If MEN2A is suspected, the diagnosis is obtained by genetic testing. Primary hyperparathyroidism is found in 20%-40% of MEN2A patients. Pheochromocytoma develops in about 50% of the MEN2A patients. It is bilateral in 50% of the cases.³ Physical features alone may trigger the suspicion of MEN2B in children. MEN2B patients have a marfanoid habitus with swollen lips; tumors of the mucous membranes of the eye, mouth, tongue, and nasal cavity; enlarged colon; and skeletal abnormalities. Hyperplasia or adenoma of the parathyroid is rare in MEN2B patients.¹

At present, surgery is the only curative treatment for MTC, whether it is sporadic, familial or part of one of the MEN syndromes. Prophylactic total thyroidectomy has proved an effective management strategy to prevent (metastatic) MTC. It improves outcome in patients with a high risk of MTC and diminishes the risk for possible recurrence after thyroid surgery. Complications of thyroidectomy may be parathyroid damage, eventually followed by hypocalcemia (due to hypoparathyroidism) and laryngeal nerve damage.⁴

Postoperative serial serum calcitonin levels should be monitored in all patients undergoing thyroidectomy for MTC to detect persistent or recurrent disease. Serum calcitonin is a well-established sensitive and specific marker of (persistent) disease.⁵ The aim of this study was evaluation of the mortality and morbidity in children and young adults treated in the Emma Children's Hospital AMC for MTC during childhood (the treatment of which may have been prophylactic).

Methods

All consecutive children (age 0 to 21 years) treated for (suspected) MTC in the Emma Children's Hospital AMC of the Academic Medical Centre (EKZ-AMC), in the period 1960 to 2005, were evaluated with regard to the course of the disease, possible complications and late effects caused by MTC and/or thyroidectomy or other treatment modalities administered for MTC. First, a checklist was made. A patient chart investigation was performed using this checklist. Data were analyzed using Statistical Package for the Social Sciences (SPSS), SAS and MS Excel software. Descriptive statistics were calculated using frequencies.

Results

In total, 11 children were treated for (suspected) MTC in our hospital. Ten patients were diagnosed as having MEN syndrome. Nine children, belonging to 3 MEN2A families, were diagnosed as having the MEN2A-syndrome, of whom 8 underwent thyroidectomy at a median age of 13 years (range 7 to 22). One of these 9 patients did not yet undergo thyroidectomy and is yearly screened by measuring pCT and bCT. The patient is 21 years old and the levels of pCT and bCT are still in the normal range. One patient was diagnosed as having the syndrome of MEN2B. This patient underwent thyroidectomy at the age of 16.

The reason for screening or genetic analysis in these ten patients was a known form of MTC in the family. In six patients, genetic analysis was done and all nine patients had aberrant basal or pentagastrin-stimulated calcitonin at thyroidectomy.

One patient, with non-MEN MTC, underwent thyroidectomy at the age of 8. This patient underwent a genetic analysis in a study involving one hundred

Patient no.	RET-mutation	Age at thyroidectomy (years)	Pathological findings in thyroid	CT at last follow-up (mg/L)
1	Cys634Arg	7	MTC (T ₄ N ₁ B ₁ M ₁)	44.00
2	Cys620Arg	8	C-cell hyperplasia	0.06
3	Cys618Ser	12	MTC (T ₁ N ₀ M ₀)	0.08
4	Cys618Ser	13	C-cell hyperplasia	0.12
5	Cys634Gly	13	MTC (T ₁ N ₀ M ₀)	0.05
6	Cys618Ser	13	C-cell hyperplasia	0.04
7	-	13	MTC (T ₃ N ₀ M ₀)	0.03
8	-	16	MTC	0.44
9	-	19	MTC (T ₃ N ₁ M ₀)	19.00
10	-	22	MTC (T ₁ N ₀ M ₀)	0.24
11	Cys634Gly	Thyroidectomy has not yet taken place (Current age: 21 year)	-	0.08

Table 1. Summary of most important characteristics of patients included in this study

red patients with Hirschsprung's disease and underwent a thyroidectomy as a result of positive screening for the RET-*proto-oncogene*.

The histological findings were MTC with C-cell hyperplasia in 7 patients and C-cell hyperplasia alone in 3.

At the last follow-up, four patients had increased basal CT and CEA levels (median age at thyroidectomy: 17.5 years, range 7 to 22), indicating persistent disease. Three of the four patients with raised calcitonin levels during follow-up underwent re-operation with lymph node dissection confirming metastatic disease.

Three patients had a complication of thyroid surgery in the way of permanent hypoparathyroidism (n=2) or permanent recurrent nerve damage (n=1).

Conclusion

In conclusion, all patients diagnosed with MEN-syndrome were alive at follow-up. However, 4 of the 11 patients (36 %) had metastatic MTC at the last follow-up. Two patients had definitive hypoparathyroidism and one patient had definitive recurrent nerve damage as a consequence of thyroid surgery.

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Low Mannose-binding Lectin (MBL) levels in neonates with pneumonia and sepsis

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Introduction

Neonatal sepsis and pneumonia are important causes of morbidity and mortality in neonates admitted to the Neonatal Intensive Care Unit (NICU).¹ Mannose-binding lectin (MBL), a plasma protein that plays an important role in the innate immune defense, might play a role. After binding to various micro-organisms, MBL activates the lectin pathway of the complement system, which leads to opsonization and enhanced phagocytosis.² Circulating MBL plasma levels and functional activity are associated with three polymorphisms (termed *X/Y*, *H/L*, and *P/Q*) in the promoter region of the *MBL2* gene, as well as three structural mutations (together termed the *O* variant) in codon 52, 54 and 57 of exon-1.³ The wild-type allele is called *A*. Variant *MBL2* genotypes (corresponding to the *XA/XA*, *A/O*, and *O/O* haplotypes) are associated with reduced or deficient MBL plasma levels.³ MBL deficiency is seen in approximately 40% of the European population⁴, and has been associated with an increased susceptibility to infections, especially in children and immunocompromized individuals.^{5,6} In neonates, low gestational age is also associated with low MBL levels.^{7,8} We investigated whether MBL deficiency is associated with the presence of early-onset neonatal sepsis and severe late-onset neonatal infections.

Material and methods

We determined MBL plasma levels at birth by ELISA technique in neonates admitted to the NICU of the Emma Children's Hospital. Sixty-seven *MBL2* genotypes were detected by Taqman analysis. Based on clinical and laboratory parameters during the first 72 postnatal hours, children were designated to an early-onset sepsis (EOS), possible EOS, or no EOS group. We also recorded the occurrence of severe late-onset infection (LOI), defined by culture-proven pneumonia or sepsis between 72 hours and 30 days after birth. The association between MBL plasma level and EOS and LOI was studied using the Kruskal-Wallis and Mann-

Whitney U tests, respectively. The association between MBL deficiency and EOS was studied by ordinal logistic regression, with the three EOS categories with increasing severity as the ordinal outcome variable. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). The OR can be interpreted as an estimation of the relative risk of being in a higher EOS category. The impact of MBL deficiency on the occurrence of LOI was analyzed with the chi-square test and expressed in crude relative risks (RR) and their 95% CIs.

Results

The median (range) gestational age of the 85 neonates was 32⁺ (27⁺ - 42⁺) weeks. Thirty-one (37%) neonates were admitted for reasons other than suspected infection (no EOS group). In the remaining 54 neonates infection was suspected and antibiotic treatment was started. Of these, 40 (47%) had a possible EOS, and 14 neonates (16%) appeared to have EOS. The median (range) MBL plasma levels were 1.72 (0.08-4.16) µg/mL, 0.77 (0.02-3.69) µg/mL, and 0.38 (0.01-3.63) µg/mL, respectively ($p=0.03$, Fig. 1a). For neonates with deficient MBL plasma levels (previously determined to be ≤ 0.7 µg/mL⁹), relative to neonates with sufficient levels, the OR of being in a higher early-onset sepsis category was 1.7 (95% CI: 1.0-2.9, $p=0.06$). This OR was 1.8 (95% CI: 1.0-3.3 for neonates with variant *MBL2* haplotypes ($n=25$) compared to neonates with wild-type *MBL2* haplotypes ($n=42$) ($p=0.06$). The median (range) MBL plasma level at birth was 1.11 (0.02-4.16) µg/mL in 75 neonates without LOI, compared to 0.42 (0.01-2.10) µg/mL in 10 neonates with LOI ($p=0.01$, Fig. 1b). Eight out of ten neonates with LOI had deficient MBL plasma levels at birth. The crude RR for developing LOI was 5.7 (95% CI: 1.3-25.3) for the neonates with deficient compared to sufficient MBL plasma levels at birth ($p=0.01$). The crude relative risk for developing LOI was 2.8 (95% CI: 0.7-10.7) for neonates with variant compared to wild-type *MBL2* haplotypes ($p=0.14$). In total, 21 out of 85 neonates had experienced either an EOS or a LOI during the entire study period. Their median (range) MBL plasma level was 0.43 (0.01-3.63) µg/mL, compared to 1.12 (0.02-4.16) µg/mL in neonates without infection ($p=0.02$, Fig. 1c). The RR for developing either EOS or LOI was 2.3 (95% CI: 1.1-5.0) for neonates with deficient MBL plasma levels at birth ($p=0.04$), and 1.9 (95% CI: 0.8-4.3) in neonates with variant *MBL2* haplotypes ($p=0.15$).

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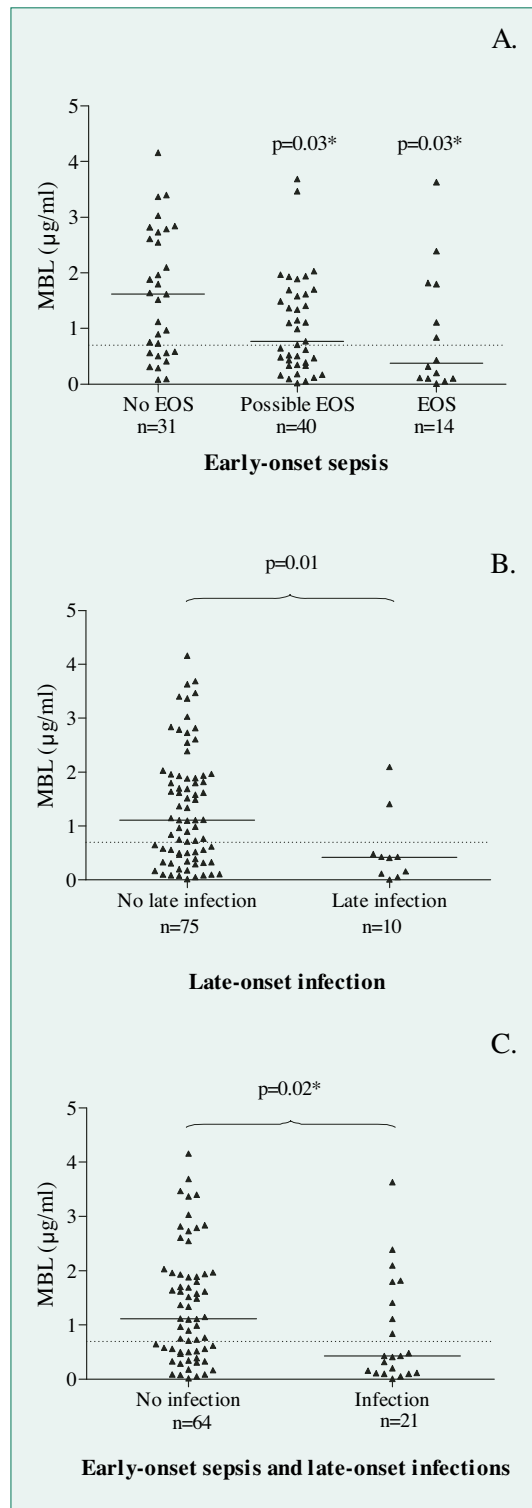


Figure 1. Infections and MBL plasma levels of 85 neonates. Dotted lines represent MBL plasma levels of 0.7 µg/mL. *P values between group below and far left (no infection) group.

- A. No early-onset sepsis (EOS) versus possible EOS and versus EOS;
- B. No late-onset infection (LOI), defined as sepsis and pneumonia, versus LOI;
- C. No infections during the entire study period versus either early-onset sepsis or late-onset infections.

Discussion

NICU-patients with EOS or LOI had statistically significant lower MBL plasma levels at birth than neonates without these infections. These data suggest that MBL might be involved in the protection against neonatal infections. Consumption does not seem to be the reason for these low MBL levels. Instead, low levels are associated with low gestational age and variant MBL2 haplo-

types.⁷ Indeed, neonates with variant MBL2 haplotypes also had an increased OR of being in a more severe EOS category than neonates with wild-type MBL2 haplotypes (p=0.07).

The association between severe LOI (pneumonia and sepsis) and low MBL plasma levels at birth needs to be further investigated: some of these neonates might have developed sufficient MBL plasma levels before the onset of infection and might therefore have had sufficient levels for protection against micro-organisms. The lack of statistical association between variant MBL2 haplotypes and LOI might be explained by insufficient statistical power. A case-control study might solve these problems.

In contrast, the only two studies on MBL and neonatal sepsis did not report an increased infection risk in neonates. However, our cohort consisted of neonates with gestational ages ranging from 28-42 weeks, while the other consisted of extreme premature neonates (<32 weeks⁸ and very-low-birth weight⁹) who are generally more prone to infections.¹ Furthermore, Ahrens *et al.* probably underestimated the effect of low MBL plasma levels by defining MBL deficiency as the presence of exon-1 mutations.⁹ He missed the effect of low MBL plasma levels due to prematurity and XA haplotypes. On the other hand, Hilgendorff *et al.* did not find decreased MBL plasma levels in extreme premature neonates with congenital sepsis.⁸ However, this was a small cohort and therefore not necessarily excludes a possible association between MBL deficiency and sepsis.

In conclusion, our results suggest that MBL deficiency may be a risk factor for neonatal infections. Possibly, the use of prophylactic antibiotics can be reduced in neonates with sufficient MBL plasma levels. Furthermore, MBL substitution therapy might be considered for prevention or treatment of severe neonatal infections.

Acknowledgements:

We would like to thank Charlotte Dorrepaal for collecting clinical data and plasma samples and Joris van der Post for helpful discussion.

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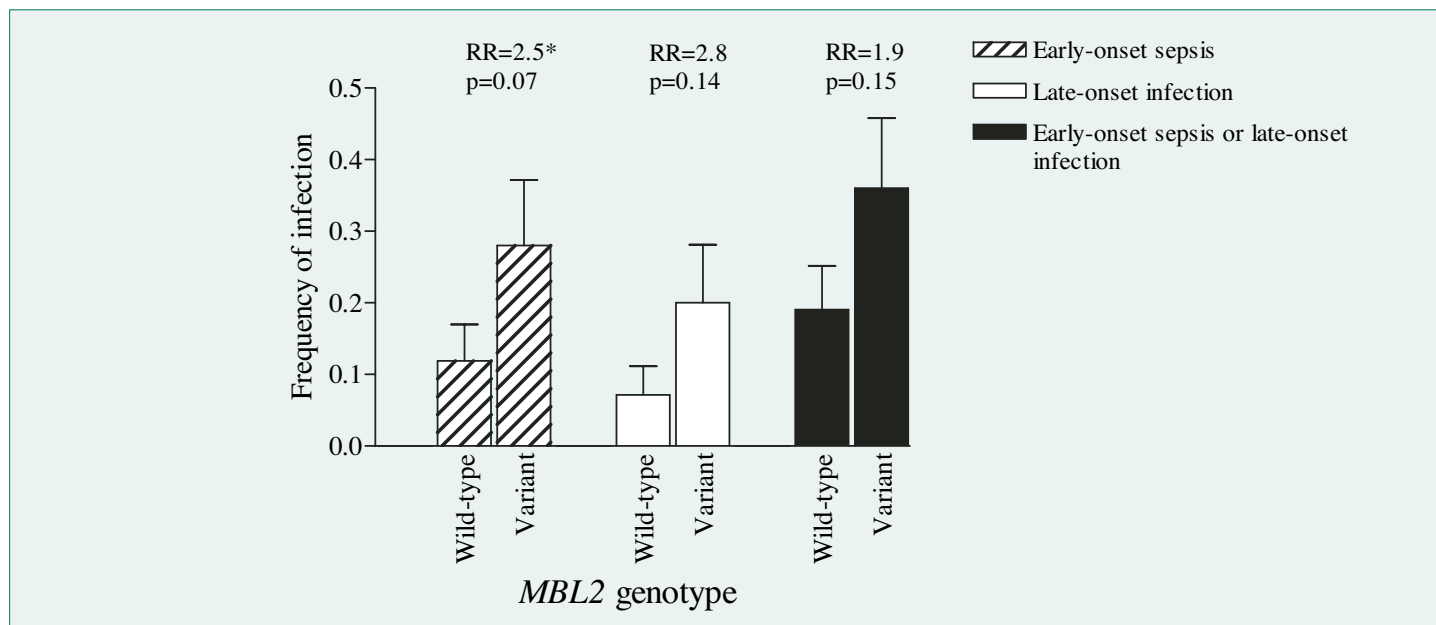


Figure 2. MBL2 genotype and infection in 67 neonates. Frequency of early-onset sepsis, late-onset infection or both. Crude relative risks (RR) of neonates with variant MBL2 haplotypes compared to wild-type MBL2 haplotypes are shown.

* Early-onset group compared to no early-onset group.

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Idiopathic ketotic hypoglycemia is associated with an impaired gluconeogenesis: an *in vivo* stable isotope study

H. H. Huidekoper¹, Th. Westphal¹, M. Duran^{1,2}, M.T. Ackermans³, H.P. Sauerwein⁴ and F.A. Wijburg¹

Introduction

Idiopathic ketotic hypoglycemia (IKH), also named “toddler hypoglycemia”, is the most common cause of hypoglycemia in childhood.¹ IKH is characterized by recurrent episodes of hypoglycemia with high ketone levels in plasma and urine, provoked either by a period of fasting often in combination with intercurrent illness or a ketogenic, low-caloric diet.² The pathogenic mechanism in IKH has not been fully elucidated. An insufficient gluconeogenesis due to a limitation in precursor supply has been suggested, as the plasma levels of gluconeogenic amino acids, especially alanine, were found to be decreased in IKH patients.³ Others have argued that IKH is not a true disease, but represents the lower tail of the Gaussian distribution of fasting tolerance in children.⁴ This is supported by the observation that hypoglycemia may also develop in a subset of healthy children after a relatively short fast.⁵ Beyond the age of six a spontaneous remission is usually seen.

To obtain more knowledge about the glucose kinetics in IKH during fasting, the rate of endogenous glucose production, derived from glycogenolysis and gluconeogenesis, and the rate of glucose utilization were quantified in four IKH patients during a fasting test using *in vivo* stable isotope techniques.^{6,7}

Materials and Methods

Subjects: Four children aged 2.8 – 6.7 yrs with a history of at least one documented episode of ketotic hypoglycemia of unknown etiology, in whom a fasting test was performed to evaluate their fasting tolerance, were included in our protocol. Extensive metabolic evaluation, including organic acid analysis in urine, plasma acylcarnitine profiling and plasma amino acid analysis, as well as a full endocrinologic evaluation did not reveal a metabolic or endocrine disorder in any of the patients. All parents or legal guardians of the patients gave informed consent prior to the studies. The study was approved by the Institutional Review Board. Demographic data on the patients are summarized in Table 1.

Study design: All subjects were admitted one day before the fasting test. Fasting was started after the consumption of a carbohydrate-rich meal in the late afternoon. Thereafter all subjects were given deuterium-enriched water (99% pure; Cambridge Isotope Laboratories, Cambridge, MA) at a dose of 5 g per kg body water (assumed to be 65% of total body weight) in

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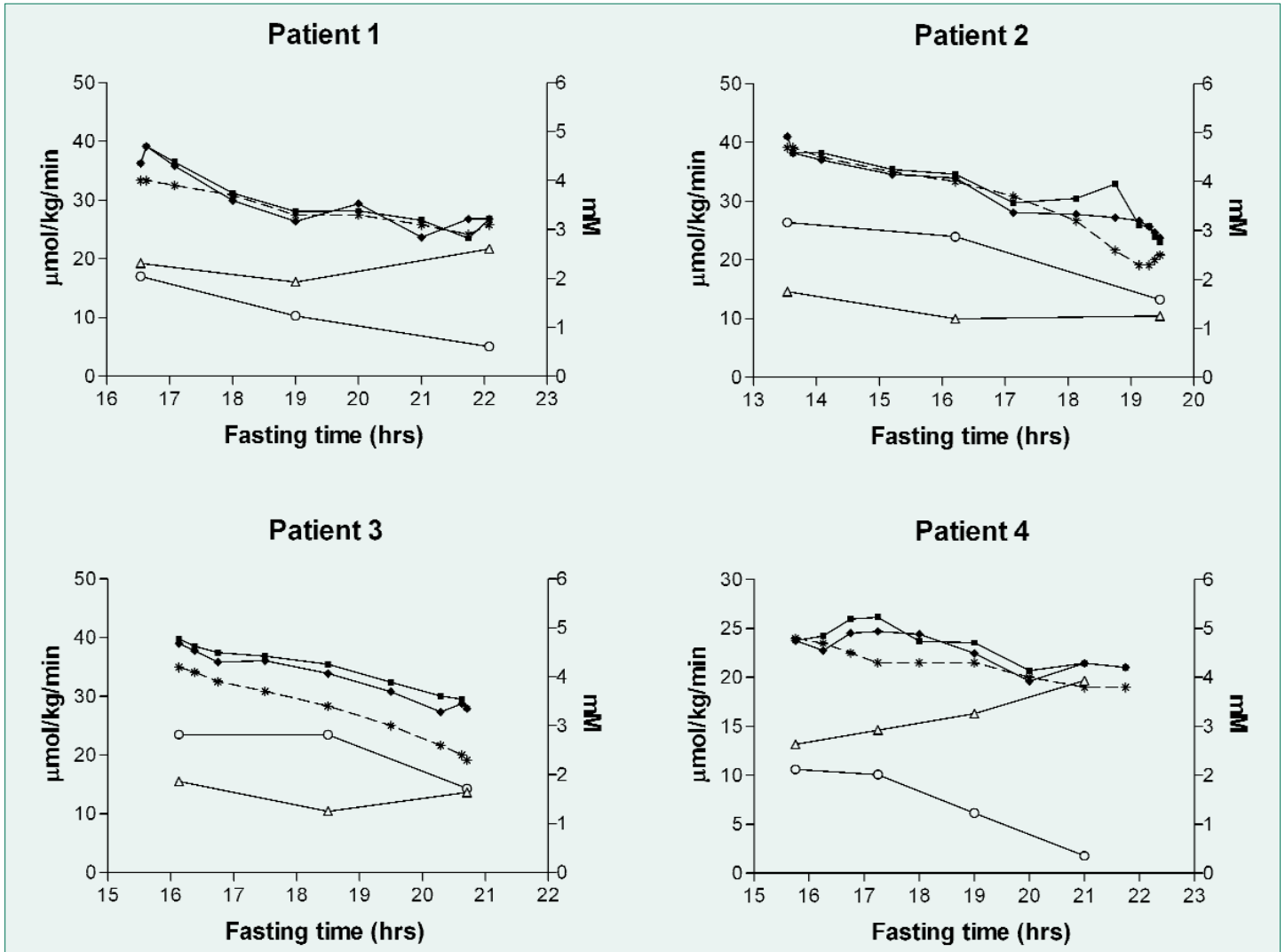


Figure 1. Glucose kinetics during fasting in all patients. Plasma glucose (T, dashed line) is plotted on the right y-axis in mmol/L. Ra glucose (u), Rd glucose (n), GGL (O) and GNG (Δ) are plotted on the left y-axis in μmol/kg/min.

5 doses to determine fractional gluconeogenesis.⁸ At 8 am the next morning a continuous infusion (0.22–0.67 μmol/kg/min, depending on the age of the patient) of [6,6-²H₂]glucose (99% pure; Cambridge Isotope Laboratories, Cambridge, MA) was started after administration of a priming dose (17.6–52.7 μmol/kg, depending on the age of the patient). At regular time intervals blood samples were drawn and centrifuged after which the plasma was stored at -20°C until analysis. Blood glucose levels were checked regularly. The test was either terminated when the blood glucose level was ≤ 2.5 mmol/L and/or when clinical symptoms of hypoglycemia occurred or after 22 hrs of fasting. After cessation of the test subjects were immediately given carbohydrate-rich drink and a meal.

Blood Sample Analysis: Plasma glucose was determined in all blood samples. Blood ketones (acetoacetate + 3-hydroxy-butyrate), glucoregulatory hormones, free fatty acids (FFA) and alanine levels were determined in various blood samples during the test. Plasma [6,6-²H₂]glucose enrichment, deuterium enrichment in glucose at position C₅ and deuterium enrichment in plasma were analyzed with gas chromatography / mass spectrometry.⁸

Calculations and statistical analysis: The rate of endogenous glucose production (R_a glucose) and the rate of glucose utilization (R_d glucose) were calculated with Steele's non-steady-state equations.⁹ The absolute gluconeogenesis

(GNG) was calculated by multiplying R_a glucose by the fractional gluconeogenesis. Fractional gluconeogenesis was calculated as follows: 100% × (deuterium enrichment in glucose at position C₅ / deuterium enrichment in plasma).⁸ The absolute glycogenolysis (GGL) was calculated by subtracting GNG from R_a glucose. Spearman correlation coefficients were calculated for the association between R_a glucose and plasma glucose, and R_a glucose and R_d glucose in all patients.

Results

Plasma values: At baseline (after overnight fasting) plasma glucose levels were normal in all four patients. Patients 1, 2 and 3 became hypoglycemic after approximately 20 hrs of fasting. Patient 4 was able to complete the whole test without hypoglycemia (Table 2). At the end of the test all patients showed an appropriate hormonal response with undetectable insulin levels and elevated glucagon, cortisol, epinephrine and norepinephrine levels (data not shown). FFA levels increased in all subjects during fasting. Plasma ketone levels were markedly elevated in all patients at the end of the test. Plasma alanine levels decreased during fasting in patients 1, 2 and 3, but increased in patient 4 (Table 2).

Glucose kinetics (Figure 1): At baseline the rates of endogenous glucose production (R_a glucose) were in the normal range for each age group.⁶ R_a glucose decreased in all patients during fasting and showed a good correlation with plasma glucose levels (R was 0.604 to 0.933). The rate of glucose utilization (R_d glucose) showed a near perfect correlation with R_a glucose during the whole test in all patients (R was 0.900 to 0.965). The absolute glycogenolysis (GGL) decreased in all patients during fasting. The absolute gluconeogenesis (GNG) increased marginally in patient 1 and decreased in patients 2 and 3 during fasting. In patient 4 GNG increased substantially during fasting.

Subjects	Sex	Age (y)	Height (m)	Weight (kg)
Patient 1	F	2.8	0.93 (-0.5 SD)	13.0 (-0.7 SD)
Patient 2	M	3.3	1.01 (0 SD)	16.5 (0 SD)
Patient 3	M	3.9	1.05 (0 SD)	16.0 (-0.7 SD)
Patient 4	M	6.7	1.22 (-1.2 SD)	22.4 (-0.2 SD)

Table 1. Subject characteristics

Subjects	Glucose (mmol/L)		FFA (mmol/L)		Ketones (mmol/L)		Alanine (μmol/L)	
	Baseline	End test	Baseline	End test	Baseline	End test	Baseline	End test
Patient 1	4.0	2.8	1.19	2.73	1.06	5.17	173	152
Patient 2	4.7	2.5	0.88	2.05	0.28	2.06	172	100
Patient 3	4.2	2.3	0.86	3.20	0.39	2.60	197	91
Patient 4	4.8	3.8	1.80	2.22	0.92	3.11	211	227

Table 2. Plasma values at baseline and at the end of the fasting test.

Discussion

The objective of this study was to gain more insight in the glucose kinetics during fasting in children with idiopathic ketotic hypoglycemia (IKH) and to determine if hypoglycemia in IKH develops due to a decrease in endogenous glucose production or an increase in peripheral glucose utilization. Our data show that in IKH hypoglycemia develops because of the inability to maintain an adequate endogenous glucose production during fasting and not because of an increase in peripheral glucose uptake. As endogenous glucose production is the sum of glycogenolysis and gluconeogenesis it is important to differentiate between these two pathways. In all subjects a significant decrease in GGL was seen during fasting, indicating glycogen depletion. However, the significant decrease in GGL was not accompanied by a significant increase in GNG in the patients who became hypoglycemic during the test despite the increased FFA, glucagon and epinephrine levels, which are all important stimuli of the gluconeogenesis. As glycogen is slowly depleted during fasting gluconeogenesis should be up-regulated to maintain normoglycemia. Therefore, it appears that in IKH patients become hypoglycemic because of an inadequate gluconeogenesis. It has previously been demonstrated that there is no enzyme defect in the gluconeogenic pathway in patients with IKH.¹⁰ The possibility of a limitation in amino acid supply, especially alanine, for gluconeogenesis in IKH has been suggested.³ However, this has been questioned by others.¹¹ In the present study plasma alanine levels decreased in patients 1, 2 and 3, whereas plasma alanine remained stable in patient 4 who was able to maintain normoglycemia. This decrease in alanine was most pronounced in patients 2 and 3, who showed a marked decrease in gluconeogenesis during the test. This does suggest that a limitation in the supply of alanine compromises gluconeogenesis in IKH. As patients get older this limitation may be overcome, as suggested by the results in patient 4. In conclusion, we demonstrate that hypoglycemia in IKH is caused by an inadequate production of glucose because

of the inability of gluconeogenesis to compensate for the depletion of glycogen during fasting. A limitation in the supply of alanine for gluconeogenesis seems implicated in IKH.

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Late effects on cardiac function in pediatric septic shock survivors

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Introduction

Septic shock (SS) is a life-threatening disease, characterized by impaired myocardial contractility, loss of vascular tone, and capillary leakage leading to diminished organ perfusion and the development of multiple organ system failure. Treatment consists of adequate fluid replacement and the administration of vasoconstrictive agents (VA) such as dopamine and norepinephrine.^{1,2} Myocardial dysfunction is one of the key factors in the development of circulatory failure during septic shock and is considered to be caused by hypoperfusion of myocardial tissue and cardio-depressant properties of circulating substances (TNF- α , IL-1 and other cytokines).³ Myocardial cell death might also play a role as cardiac troponin is elevated in patients with SS.^{4,6} Moreover, the effect of VA is mainly attributed to their vasoconstrictive effect on the circulation. This effect may compromise the microcirculation in capillary beds and may lead to aggravation of myocardial damage and a further decrease of myocardial function.^{7,9}

From this point of view one can hypothesize that both the underlying illness (i.e. septic shock) and the side-effects of its treatment with VA may result in permanent damage of the developing heart in children. However, follow-up studies of patients with SS have mainly focused on mortality and short-term cardiac morbidity.¹⁰ In addition, there are no studies on the long-term effects of SS and the consequences of prolonged administration of VA on the growing myocardium in children.

To test this hypothesis, we conducted a pilot study in meningococcal septic shock survivors to evaluate cardiac function with echocardiography and electrocardiography during rest and exercise. Twenty-two patients met inclusion criteria (e.g. age >12 years at follow-up). Episodes of ventricular extrasystole were found in 3 patients during exercise testing and decreased left ventricular function was found in 1 patient. Mean age at time of the septic shock episode was 9 years; mean follow-up time was 7 years.

To date, patients with meningococcal SS present at a younger age, whereas treatment nowadays is much more aggressive. We hypothesize that younger children might be even more susceptible to permanent damage of the growing cardiovascular system than the studied patient group. We therefore performed the present study.

Methods

This historical cohort study was conducted on our 12-bed tertiary PICU at the Emma's Children's Hospital AMC of the University of Amsterdam. SS-survivors admitted to the PICU between 1995 and 2004 were included if they had received ≥ 24 hours' administration of VA. Exclusion criteria were cardiac disorders, cardiogenic shock and severe psychomotor retardation.

In our outpatient clinic cardiac performance was evaluated by history and physical examination, 24-hours-ECG registration, echocardiography and electrocardiography (ECG), at rest and during exercise, in patients over 7 years of age.

Age at admission, age at follow-up study, severity of illness (PIM II score), and length of PICU stay were evaluated.

For all statistics, SPSS for Windows (Version 11, 1, SPSS Inc.) was used. The Mann-Whitney test was used for continuous variables and the chi-squared test was used for categorical variables.

Results

Of 123 eligible patients, 89 were evaluated, 10 patients declined, and 24 could not be located. No statistically significant differences were found between participating patients and non-participating patients. (Table 1) None of these 89 patients had cardiac complaints and none showed cardiac abnormalities at physical examination. In 14 of these children abnormalities were detected: episodes of ventricular extrasystole during and after exercise ($n=3$), rhythm disturbances on 24-hours-ECG ($n=2$), mild left ventricular hypertrophy ($n=1$) and mild systolic dysfunction of the right ventricle ($n=8$) on echocardiography. No statistical differences were found between patients that had cardiac abnormalities and patients that had no cardiac abnormalities, except for gender. Of the children with cardiac abnormalities more children were boys ($p<0.05$). (Table 1)

Discussion

In conclusion, the results of this historical cohort study show a 15% prevalence of ventricular dysrhythmia and mild systolic dysfunction in 90 SS-survivors, 1-10 years after admission.

Myocardial dysfunction due to hypoperfusion, cardio-depressant substances, myocardial cell death and myocarditis, is one of the key factors in the development of circulatory failure in septic shock.³ The vasoconstrictive effect of catecholamines on the circulation may compromise the microcirculation in distinct capillary beds and may lead to aggravation of myocardial damage and further decrease of myocardial function. However, some

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	Non-participating SS-survivors	Participating SS-survivors	Normal cardiac function	Abnormal cardiac function
Number of patients n	34	89	75	14
Age at SS-episode yrs	4.4	2.8	2.7	2.2
Age at follow-up yrs	—	8.7 (2.4-20.4)	8.8 (2.4-20.4)	8.9 (4.1-13.8)
Time since discharge yrs	—	6.1 (0.5-10)	5.6 (0.8-10)	6.6 (1.2-9.1)
Sex ♂/♀	12 ♂/22 ♀	51 ♂/38 ♀	39 ♂/36 ♀	12 ♂/2 ♀ *
PIM-II %	6.8	7.8	7.5	8.6
Length of stay PICU days	5.0 (1-19)	5.0 (1-35)	5.0 (1-35)	5.5 (2-45)
*P<0.05				

Table 1. Patient characteristics (median, range)

studies report that catecholamines play an important factor in vascular remodeling and that norepinephrine influences outcome favorably.^{7,9} Theoretically, myocardial dysfunction could be permanent after septic shock which may lead to heart failure and rhythm disturbances. The long term consequences of the myocardial dysfunction and ventricular rhythm disturbances we found in this study are virtually unknown. Persisting rhythm disturbances could be a risk for sudden cardiac death and systolic myocardial dysfunction could lead to heart failure. Therefore it is important to evaluate myocardial function prospectively in the acute phase of SS and thereafter at regular time intervals.

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Clinical implications of mutation analysis in Primary Hyperoxaluria Type 1

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Introduction

Primary Hyperoxaluria type 1 (PH1) is a rare metabolic disorder with an autosomal recessive pattern of inheritance, defined by a functional deficiency of the peroxisomal liver-specific enzyme alanine glyoxylate aminotransferase (AGT). Physiologically, AGT catalyzes the conversion of glyoxylate into glycine. In the absence of functional AGT, glyoxylate is partly oxidized to oxalate and partly reduced to glycolate. The kidneys excrete these metabolic end products in the urine. Oxalate may precipitate with calcium in renal tissue, leading to urolithiasis, nephrocalcinosis, urinary tract infections and/or hematuria. Approximately 50% of the patients develop a renal insufficiency resulting in systemic oxalate depositions in various tissues, called oxalosis.¹ Conservative treatment consists of inhibition of crystallisation by administration of citrate and hyperhydration. A significant proportion (35%) of PH1 patients responds to treatment with pyri-

doxine, a co-factor of AGT, resulting in a reduction of oxalate excretion by >30%. Since even the most intensive dialysis sessions cannot prevent systemic deposition in cases of renal failure, early liver (in case of relatively preserved renal function) or combined liver/kidney transplantation is the current therapeutic strategy in these patients. Renal transplantation without concomitant liver transplantation may result in graft failure due to recurrence of oxalate depositions from mobilized calcium oxalate depositions. The diagnosis of PH1 is based on urinalysis (elevated oxalate and glycolate in urine), enzyme analysis (decreased AGT activity in a liver tissue biopsy) or mutation analysis of the AGT gene.

The AGT gene, located at chromosome 2q37.3, encodes the 392 amino acids of AGT.¹ More than 50 disease-causing mutations have been found.^{2,3} The relationship between clinical outcome and genotype has been obscure to date. In order to investigate this relationship, we performed mutation analysis in a Dutch cohort of previously characterized PH1 patients.

Patients and Methods

From a previously described cohort of 57 PH1 patients, diagnosed between

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1970 and 2000 in The Netherlands⁴, we contacted all living patients and/or their parents to participate in this study. For mutation analysis, genomic DNA was extracted from lymphocytes using standard procedures. Six sets of AGXT-specific primers were used (primer sequences available on request). Mutation analysis was performed with DNA of the probands and, when available, with DNA of the parents in order to establish the zygosity of the proband. Mutations in the AGXT gene were compared with two criteria of patient outcome: (1) pyridoxine responsiveness, defined by a decline of oxalate levels >30% and (2) renal function, which was dichotomized as either favorable (preserved) or poor (insufficient, glomerular filtration rate <60mL/min/1.73m²).

Results

Thirty-four patients, offspring of 26 families, were enrolled in the study. Clinical data are given in Table 1. Eight different mutations were found. Homozygous patients are compared in Table 2. All patients homozygous for Gly170Arg or Phe152Ile substitutions were responsive to pyridoxine therapy. In three patients, pyridoxine treatment was initiated only after kidney transplantation. All were found to be responsive to pyridoxine therapy. Isolated kidney transplants of seven patients (six Gly170Arg, one Phe152Ile) survived during a mean follow-up period of 8 (range 3-14) years. All patients homozygous for the 33insC insertion developed End Stage Renal Disease (ESRD) within the first year of life.

Discussion

Compatible with other studies, we found the Gly170Arg substitution to be the most common mutation in Dutch PH1 patients. Its association with pyridoxine responsiveness is consistent with earlier reports.³⁷ Previously, this mutation has been associated with the phenotypical combination of severe hyperoxaluria and hyperglycolic aciduria in the presence of a considerable AGT activity *in vitro* and with pyridoxine responsiveness. The biological explanation for the discrepancy between *in vitro* and *in vivo* AGT activity lies in the unique phenomenon of enzyme mistargeting. Two main polymorphic variants of the AGXT gene exist in normal European and North American populations. The functionally significant difference between the proteins encoded by the “major” AGXT allele (frequency ~80%) and the “minor” AGXT allele (frequency ~20%) is the presence of a Pro11Leu polymorphism in the latter. This mutation leads to a small extent ($\pm 5\%$) to a mistargeting of AGT to the mitochondria instead of peroxisomes. Motley *et al*³ showed that the Gly170Arg mutation, which is always located on the minor allele, results in increased mitochondrial mistargeting of AGT up to more than 90%, leading to inactivation of the enzyme *in vivo*. The mistargeting is the result of a delay of the dimerization of AGT, which prevents the folding of the protein. As a result of this, the amino terminus remains accessible for mitochondrial import. Pyridoxine possibly increases the residual activity of the 10% peroxisomal located AGT. Previously, there has been discussion whether this relatively favourable biological background (i.e. the pyridoxine responsiveness) is in line with the clinical outcome of patients with this mutation. All homozygous Gly170Arg patients in our study, who had a preserved renal function at the time of diagnosis and who were treated accordingly with pyridoxine, a high fluid intake and potassium citrate, were able to preserve renal function throughout the follow-up period. However, five homozygous patients had already developed ESRD at the time of diagnosis. Therefore, renal function can be preserved in patients with a Gly170Arg mutation after a timely diagnosis and treatment with pyridoxine. This is supported by the course after isolated renal transplantations in Gly170Arg-positive patients in our cohort. In contrast to what might be expected in PH1 patients with isolated kidney transplantation, renal function was preserved in 4 out of 6 of these patients after a median follow-up of more than 7 years, most likely as a consequence of pyridoxine therapy. The only Gly170Arg patient not responding to pyridoxine was affected by a second Val336Asp mutation which presumably changes the enzyme conformation. For the Phe152Ile genotype, the beneficial effect of pyridoxine is in line with previous observations.⁸

Total number of patients	34
Median age at time of investigation years	27 (363)
Male/female	15/18
Median age at time of diagnosis years	6.6 (050)
Urolithiasis	19
Nephrocalcinosis	17
Pyridoxine sensitivity	15
Renal insufficiency at the time of diagnosis	15
ESRD at the time of diagnosis	14
ESRD/renal insufficiency at follow-up	21
Infantile onset	8
Kidney transplantation	12 in 10 patients
Combined liver/kidney transplantation	6
Deceased	2

Table 1. Patient characteristics

Abbreviation: ESRD, end-stage renal disease

Patients with the 33insC mutation showed very little residual enzyme activity and early development of ESRD or death. The insertion of this nucleotide leads to a frame shift creating a termination codon at residue 44.

Clinical implications

This study shows that mutation analysis may contribute to an improvement of clinical decision making in PH1. The finding of any of the 3 above described mutations (Gly170Arg, Phe152Ile, and 33insC) might make the need for enzyme analysis by the invasive liver biopsy unnecessary. In addition, for patients who have already developed ESRD, mutation analysis can predict if the patient will respond to pyridoxine treatment after receiving an isolated kidney transplantation (Gly170Arg, Phe152Ile). The presence of mutations resulting in pyridoxine unresponsiveness (33insC, Gly82Arg) may favor choosing a liver transplantation or a combined liver-kidney transplantation.

Results from the whole study have been published previously.⁴

Mutations	Allele frequency	Nr of patients	PyrR	Mean AGT %
Homozygous				
Gly170Arg	43%	11	6 + ^a	35.1
Phe152Ile	19%	4	3 + ^b	11.5
33insC	15%	3	1 -	3.8
Gly170Arg, Val 336Asp ^c	5%	1	1 -	4.5
Gly82Arg	9%	2	2 -	3.6
Heterozygous				
Gly170Arg/ 33insC		1	1 +	16.3
Gly170Arg/ Phe152Ile		1	1 +	10.0
Gly170Arg/ X ₀		2	2 +	5.7
Phe152Ile/ 33insC		1	1 +	5.9
Phe152Ile/ Gly170Arg, Val336Asp		1	1 +	10.2
total number		27	19	

Table 2. AGXT mutations compared with pyridoxine responsiveness and AGT activities
Abbreviations. PyrR, pyridoxine responsiveness (i.e. $\geq 30\%$ reduction of urinary oxalate upon pyridoxine administration); +, pyridoxine responsive; -, not responsive; AGT%, percentage AGT-activity *in vitro* related to AGT-activity *in vitro* of control tissue; X₀, no mutations found on one allele

a pyridoxine was started in two patients after a kidney transplantation was performed

b pyridoxine was started in one patient after a kidney transplantation was performed

c double homozygous

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WHAT'S NEW IN PEDIATRICS

Constipation in (early) infancy and childhood: Pathogenesis and diagnostic procedures

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Defecation problems in neonates and children are common in general pediatric practice, and result in 3% of all consultations to a pediatrician. These disorders form the most common complaint in childhood gastrointestinal disease, and may constitute up to 25% of all consultations to a pediatric gastroenterologist. It is notable that in approximately 40% constipation could be traced back to the first month of life.

After birth, the passage of the first stool (meconium) occurs in 95% within 24 hours and in 99% within 48 hours in healthy term infants.^{1,2} This percentage however dramatically drops to 66% in preterm (≤ 32 weeks gestation) and low birth weight (<2500 grams) infants. In most cases the cause of this delay is unknown, although it may simply reflect impaired maturation.^{3,4} From a clinical point of view, it should alert the clinician as it may be the first sign of a defecation disorder such as Hirschsprung's disease. Therefore, early diagnosis is warranted.

We aimed to investigate the accuracy of different diagnostic procedures in the work-up of (young) infants and children with disturbed defecation. Furthermore, we aimed to clarify the pathophysiology underlying the delayed passage of meconium.

If delayed passage of meconium presents in combination with clinical findings such as vomiting, irritability and abdominal distension, intestinal obstruction due to Hirschsprung's disease may be present. This is a developmental disorder of the enteric nervous system characterized by an absence of ganglion cells along a variable distance of the distal intestine, resulting in disturbed colonic motility and the absence of the rectoanal inhibitory reflex.⁵ The rectoanal inhibitory reflex is a transient relaxation of the internal anal sphincter elicited by rectal distension. Impaired or absent relaxation of the internal anal sphincter hampers the evacuation of stool leading to severe chronic constipation as described in Hirschsprung's disease.⁶

Besides children with Hirschsprung's disease, there is a subgroup of neonates with defecation problems directly after birth.⁷ In contrast to HD, these gastrointestinal symptoms resolve in the following weeks after birth. The exact reason for these transient defecation abnormalities

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is unclear. One possibility could be that the components responsible for normal motility have not developed properly. Recently, a study demonstrated that transient defecation abnormalities may result from a delayed maturation of interstitial cells of Cajal. These cells function as pacemaker cells coordinating the electromechanical activity of the gut.^{8,9} To what extent this delayed maturation also affects the rectoanal inhibitory reflex is so far unknown. We studied the role of interstitial cells of Cajal in the inhibitory neurotransmission of the murine internal anal sphincter. The relaxation of the internal anal sphincter to electrical stimulation was measured *in vitro* in an organ bath and showed that there was no difference in relaxation of the internal anal sphincter between mice lacking interstitial cells of Cajal compared to controls. However, *in vivo* experiments showed that the rectoanal inhibitory reflex in response to rectal distension was clearly diminished in mice lacking interstitial cells of Cajal.⁴⁰ This suggests that an intact network of interstitial cells of Cajal in the internal anal sphincter is necessary for a normal rectoanal inhibitory reflex, and provides evidence that interstitial cells of Cajal may be involved in the afferent part of the rectoanal inhibitory reflex. Therefore, dysfunction or delayed maturation of interstitial cells of Cajal may lead to an impaired anal sphincter relaxation and may thus be involved in rectal evacuation disorders.

To evaluate this hypothesis, the presence of the rectoanal inhibitory reflex was determined in premature neonates with a transient delayed meconium production (> 48 hours). A normal rectoanal inhibitory reflex could be elicited in all infants studied, suggesting that delayed meconium passage in this subgroup of children is not related to the absence of a rectoanal inhibitory reflex¹¹. However, it is obvious that the rectoanal inhibitory reflex is an important reflex pattern, necessary for normal defecation. Especially in children with delayed meconium production, it is of great importance to detect possible abnormalities in the rectoanal inhibitory reflex. Clearly, this implies that the age at which the rectoanal inhibitory reflex is matured has to be identified. A normal developed rectoanal inhibitory reflex to rectal distension has been shown in term and premature infants older than 30 weeks postmenstrual age.¹² However, it is unknown if the rectoanal inhibitory reflex is matured in very preterm infants (< 30 weeks postmenstrual age). Therefore, we evaluated the maturation of the rectoanal inhibitory reflex in preterm infants (26-30 weeks postmenstrual age). The conclusion of this study was that the majority of preterm infants older than 26 weeks postmenstrual age have a normal rectoanal inhibitory reflex.¹³

Anorectal manometry in the neonate offers a non-invasive diagnostic test for identifying the rectoanal inhibitory reflex. It measures pressures in the anorectal region evaluating internal and external sphincter function. In addition to anorectal manometry, two other tests are also employed in the diagnostic work-up of patients in whom Hirschsprung's disease is suspected. During a contrast enema of the colon, a calibre change with a transition of a dilated normal colon to a narrowed aganglionic bowel is typically present. Lastly, a rectal suction biopsy is taken to evaluate cholinesterase activity and the presence or absence of enteric neurones. There has been considerable debate about the most appropriate

diagnostic approach for Hirschsprung's disease.¹⁴⁻¹⁷

Therefore, we searched the literature and conducted a systematic review to determine and compare the diagnostic accuracy between contrast enema, anorectal manometry and rectal suction biopsy in infants suspected of Hirschsprung's disease. This review showed that rectal suction biopsy was the most accurate test having both the highest mean sensitivity (93%) and mean specificity (98%). Sensitivity and specificity of anorectal manometry (91% and 94% respectively) was similar to that of rectal suction biopsy, whereas contrast enema showed a significantly lower sensitivity and specificity (70% and 83% respectively).

Subsequently to this review, we conducted a prospective study in which the diagnostic accuracy of contrast enema, anorectal manometry and rectal suction biopsy in infants suspected of Hirschsprung's disease was compared. Rectal suction biopsy had the highest sensitivity (93%) and specificity (100%), but values were not significantly different from contrast enema (76% and 97% respectively) and from anorectal manometry (83% and 93% respectively). Inconclusive test results occurred most often with anorectal manometry and were lowest with rectal suction biopsy. This suggests that rectal suction biopsy is the most accurate test to diagnose Hirschsprung's disease.

Based on our studies we would like to advise clinicians in diagnosing Hirschsprung's disease. Rectal suction biopsy proved the most accurate test with the highest sensitivity and specificity, although no significant difference was shown compared to anorectal manometry and contrast enema.

However, it should be emphasized that the optimal diagnostic approach is largely determined by age and should be different in premature infants compared to older infants. In our opinion, in term neonates and older children in whom Hirschsprung's disease is suspected, rectal suction biopsy should be the first option. A rectal suction biopsy is relatively simple, quick, and efficient and can be considered an incident-free procedure. Although anorectal manometry is non-invasive in contrast to rectal suction biopsy, movement artefacts due to agitation of the child quite often make interpretation of the manometric tracing virtually impossible. Furthermore, anorectal manometry is time-consuming and requires both extensive experience and expertise. The situation in premature infants is rather different, especially as rectal suction biopsy is often too invasive with the risk of infection and perforation. In this fragile group of children anorectal manometry proved a good but most importantly, a safe diagnostic test for Hirschsprung's disease. If anorectal manometry is not conclusive or if anorectal manometry expertise is absent, rectal suction biopsy can be performed at a later stage. Meanwhile, enemas and/or laxatives should be given to relieve colonic distension in these infants. In most cases rectal suction biopsy is possible at term age.

Based on the high specificity and sensitivity, a negative rectal suction biopsy virtually rules out the diagnosis of Hirschsprung's disease. However, if symptoms persist and clinical suspicion remains high, further evaluation is certainly necessary. If anorectal manometry expertise is present in the hospital, anorectal manometry is a good option, if not one should repeat rectal suction biopsy. In

our opinion the value of a contrast enema in the work up of Hirschsprung's disease is limited because of its fairly low sensitivity and high radiation exposure. Only if the diagnosis Hirschsprung's disease has been established, contrast enema might be helpful for the surgeon to assess the localization of the calibre change and thus the presumed length of the aganglionic segment.

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