Coll. Antropol. **28** Suppl. 2 (2004) 297–304
UCD 616-089.5:577.175.8:575.22
Original scientific paper

Cholinesterase Unit Establishment and Issuing of »Warning Cards« for Carriers of Suxamethonium Sensitive Serum Butyrylcholinesterase Variants

Branka Šurina[1], Dražen Nosso [2], Zoran Šiftar[1], Zlata Flegar-Meštrić[1] and Vera Simeon-Rudolf[3]

[1] Institute of Clinical Chemistry, Clinical Hospital »Merkur«, Zagreb, Croatia
[2] Department of Otorhinolaryngology, Clinical Hospital »Merkur«, Zagreb, Croatia
[3] Institute for Medical Research and Occupational Health, Zagreb, Croatia

A B S T R A C T

Recognition of butyrylcholinesterase (EC 3.1.1.8) variants in human serum is essential to identify patients who may be susceptible to a prolonged reaction of suxamethonium and mivacurium, short-acting muscle relaxants. Thus they can be given appropriate advice along with their relatives who may be similarly affected. Therefore, Cholinesterase Unit for detection of individuals, carriers of inherited suxamethonium sensitive butyrylcholinesterase variants was established at the Institute for Clinical Chemistry of the Clinical Hospital »Merkur«, Zagreb, Croatia. A study was conducted on sera from patients referred to the Unit. Butyrylcholinesterase variants were determined by measuring the enzyme activity and inhibition by specific inhibitors in the sera of 384 patients and of the members of seven families. Cholinesterase Unit issued »Warning Cards« to the carriers of inherited serum butyrylcholinesterase variants in order to avoid prolonged apnea that suxamethonium might cause.

Key words: *butyrylcholinesterase, serum cholinesterase, cholinesterase variants, suxamethonium, neuromuscular relaxants, warning cards*

Received for publication July 4, 2002

Introduction

Since the early thirties when two enzymes, acetylcholinesterase (AChE; acetylcholine acetylhydrolase, EC 3.1.1.7) and butyrylcholinesterase (BChE; acylcholine acylhydrolase, EC 3.1.1.8) were recognized as being capable of hydrolyzing acetylcholine, a very extensive research has been conducted on their physiological and pharmacological properties pertinent to medical practice. Acetylcholinesterase, specific for hydrolysis of acetylcholine and closely related esters, is located in nerves, muscles, erythrocytes and placenta. Butyrylcholinesterase is located in the central and peripheral nervous system, liver and serum. The function of serum BChE is the subject of several hypotheses including its role in transmission of slow nerve impulses, lipid metabolism and choline homeostasis. BChE binds organophosphorus compounds (pesticides and nerve agents) thus preventing inhibition of AChE. Also, it has been shown that BChE degrades several drugs as bambuterol, aspirin, cocaine and cocaine like local anesthetics. The results of the research of the nature of butryrylcholinesterase and its variants as well as their role in the human body have been summarized and discussed in several comprehensive reviews[1–7].

At present our principal interest is in butyrylcholinesterase in human serum and its pharmacological properties to hydrolyze the short-acting muscle relaxant suxamethonium. After suxamethonium application inherited variants of human serum butyrylcholinesterase were identified in about 65% of cases with prolonged apnea as well as in some members of their families[8–11].

Suxamethonium (succinyldicholine) is one of the muscle relaxants given to patients immediately before surgery, and it is hydrolyzed by butyrylcholinesterase to form succinylmonocholine and choline. The usual (U) butyrylcholinesterase hydrolyses 90% of dose 1.0–1.5 mg/kg of suxamethonium within 1–2 minutes, thus restoring the normal neurotransmission in the smooth muscles of the respiratory tract. Atypical (A) variant of butyrylcholinesterase has a low affinity for suxamethonium and needs more time to hydrolyze the same dose of suxamethonium. Suxamethonium binds to acetylcholine receptors, blocks the transmission of neuroimpulses causing prolonged apnea[1–8]. The time to sufficient recovery of neuromuscular function following succinylcholine or mivacurium may be from one to several hours, in extreme case up to 10 hours[9–11]. The other BChE variants, like fluoride-resistant (F), silent (S), Kalow (K) or J variant also have due to various reasons, a reduced capability to hydrolyze suxamethonium. The usual BChE together with the variants give rise to fifteen phenotypes, which can be identified by standard biochemical methods coupled with family studies[2,7,9,10]. With our experience and research on cholinesterase[12,13] as well as after our participation in the »Cholinesterase Proficiency Program No 4« (the International Program for Quality Control of Measurements of Serum Cholinesterase Activity and Phenotyping, organized by R. T. Evans from St. James's University Hospital, Leeds, UK, from 1993 to 1997)[14,15], we decided to establish the first Cholinesterase Unit in our country for detection of individuals, carriers of atypical and other suxamethonium sensitive butyrylcholinesterase variants. In some Western countries similar units are being set up to issue warning cards to the patients and to establish a database of persons with inherited butyrylcholinesterase variants.

The aim of this report is to present our Cholinesterase Unit and results obtained by phenotyping 384 patients and 7 of their families as well as the need to issue

»Warning Cards« to the carriers of suxamethonium sensitive BChE variants.

Patients were from the Department of Otorhinolaryngology of the Clinical Hospital »Merkur«, Zagreb.

Cholinesterase unit

Formation, organization and research of the Cholinesterase Unit at the Institute of Clinical Chemistry, Merkur Clinical Hospital, Zagreb, Croatia was started in 2000. The main objectives of the Cholinesterase Unit are: (a) to establish the first center for detection of individuals and their families, carriers of an unusual phenotype and/or genotype with butyrylcholinesterase variants, (b) to issue »Warning Cards« (in Croatian and English) to individuals with butyrylcholinesterase variants sensitive to suxamethonium and mivacurium, (c) to establish a database of family indexes for persons from the Republic of Croatia with inherited butyrylcholinesterase variants, considering experiences of many other European countries and on the basis of standard methodologies, (d) to organize the internal and external laboratory control of measuring cholinesterase activity and phenotyping, (e) to contact and invite anesthesiologists (from Zagreb and other medical centers and hospitals in Croatia) to report patients with long periods of apnea after suxamethonium or mivacurium administration and ask them to send blood samples for detection of BChE variants, (f) to obtain reliable and accurate information about the diagnosis, medical treatment, anesthetic agents and information on relatives, (g) to introduce DNA analysis and BChE genotyping of blood samples.

Patients and Methods

We examined 384 patients ranging in age 3–29 years, undergoing tonsillectomy, and 7 families of the patients who were carriers of atypical or other suxamethonium sensitive BChE phenotypes. Venous blood was collected into vacutainer tubes (Becton Dickinson) without anticoagulant. Estimation of butyrylcholinesterase activity and inhibitor numbers was done either on the fresh serum or on the serum stored at –20 °C before further use.

BChE activity was measured in sera with 7.0 mM butyrylthiocholine as substrate according to the spectrophotometric method described by Das and Liddell[16] using the Herbos diagnostics kit (Herbos, Sisak, Croatia). The product of substrate hydrolysis, thiocholine, reacts with DTNB reagent (5,5'-dithiobis-2-nitrobenzoate) yielding a yellow anion 5-thio-2-nitrobenzoate. The increase in absorbance was followed for several minutes at 415 nm. The enzyme activity was expressed in the international unit (U/L) that corresponds to micromoles of substrate hydrolyzed per minute per liter of serum.

Phenotyping of BChE was done by measuring the inhibition of butyrylthiocholine hydrolysis by dibucaine, sodium fluoride, urea or Ro 02-0683 (dimethylcarbamate of (2-hydroxy-5-phenyl-benzyl)-trimethylammonium bromide)[16–21]. The activity of enzyme was measured immediately after the addition of dibucaine, sodium fluoride or urea and their final concentrations were 0.10 mM, 0.19 mM and 5.0 M, respectively. To determine the inhibition by R0 02-0683 serum was incubated with 32 nM inhibitor for two hours before the substrate was added. From the degree of inhibition the inhibitor numbers (percent of inhibition) DN, FN, UN and RoN for dibucaine, sodium fluoride, urea and Ro 02-0683 were calculated. BChE activity was measured on the VP ABBOTT bichromatic analyzer at 30 °C[22]. We standardized and controlled our methodology during our participation in the International Proficiency Program No 4. for quality control of measurements of se-

rum BChE activity and phenotyping provided in a period from 1993 to 1997[14,15].

The long-term analytical imprecision for the BChE activity expressed as coefficient of variation was 1.5% and 2.5% for normal and pathological activities, respectively, and analytical inaccuracy expressed as the bias for normal and pathological activities was 2.5%[12].

Results

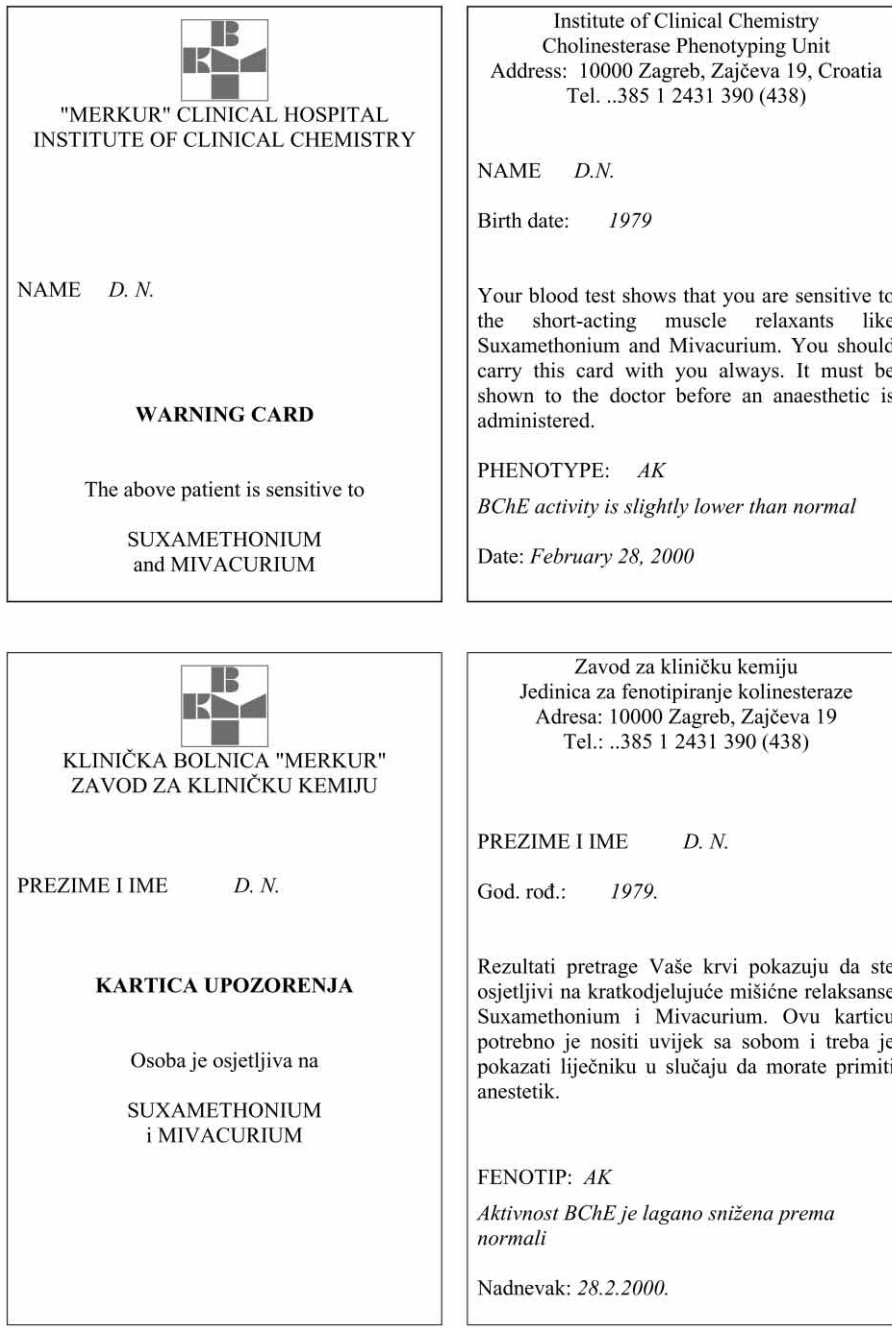
The frequency distribution of BChE phenotypes determined on the basis of activities and inhibitor numbers measured in serum taken from patients, prior to their undergoing tonsillectomy, is shown in Table 1. The frequency of usual BChE phenotype (Table 1) is similar to that presented in our previous studies of the Croatian population[12,13,23]. Ranges of BChE activities (U/L) for UU, UA, AA, UF and AK phenotypes agree with those obtained on 720 healthy individuals[12] whom we previously investigated using the same methods[22]. Activities of UA, AA, AK and AS phenotypes are significantly lower than UU and UF. This is consistent with other reports[2,12,13,20]. The US and AS phenotypes were detected which had not been observed in our previous cohorts[12,13,23].

Though we have found individuals with the A, F, S or K variants (UA, UF, US, AA, AK and AS) in 25 out of 384 patients (7%), we were only able to examine 7 families, 4 families of UA carriers and 1 family of each AS, US and AA carriers (Table 2). So far we have issued »Warning Cards« (Figure 1.) to two sisters of whom one was assigned as an AK, and another a UA phenotype carrier. For patients with suxamethonium sensitive variants (Table 2) we did not officially receive documented reports but have got only personal communications from medical doctors in charge that the patients had signs of prolonged apnea following suxamethonium applications.

TABLE 1
DISTRIBUTION OF SERUM BCHE PHENOTYPES AND THE CORRESPONDING ACTIVITIES AND INHIBITOR NUMBERS IN SERA OF 384 PATIENTS

BChE phenotype	Number identified	Frequency (%)	Inhibitor numbers ± SD				Activity ± SD U/L
			DN	FN	UN	RoN	
UU	359	92.8	81±1 (77–87)	53±3 (46–58)	45±3 (40–51)	96±1 (92–98)	7,523±1,601 (3,990–16,972)
UA	14	4.0	69±3 (64–74)	58±2 (54–62)	57±4 (53–67)	79±3 (74–83)	5,857±1,035 (4,284–7,420)
UF	5	1.4	76±2 (73–78)	45±2 (42–47)	65±2 (63–67)	94±1 (94–95)	6,651±915 (5,650–7,640)
US	3	0.9	84±5 (81–89)	52±5 (47–56)	45±2 (43–47)	98±2 (96–100)	3,002±2,222 (555–4,892)
AA	1	0.3	22	85	97	11	2,868
AK	1	0.3	59	58	62	66	3,960
AS	1	0.3	23	78	92	15	1,111
Total	384	100.0					

DN: dibucaine number; FN: fluoride number; UN urea number; RoN: Ro 02-0683 number. BChE activities and inhibitor numbers are presented as the means with the standard deviation (SD) and ranges in brackets.



"MERKUR" CLINICAL HOSPITAL
INSTITUTE OF CLINICAL CHEMISTRY

NAME *D. N.*

WARNING CARD

The above patient is sensitive to

SUXAMETHONIUM
and MIVACURIUM

Institute of Clinical Chemistry
Cholinesterase Phenotyping Unit
Address: 10000 Zagreb, Zajčeva 19, Croatia
Tel. ..385 1 2431 390 (438)

NAME *D.N.*

Birth date: *1979*

Your blood test shows that you are sensitive to the short-acting muscle relaxants like Suxamethonium and Mivacurium. You should carry this card with you always. It must be shown to the doctor before an anaesthetic is administered.

PHENOTYPE: *AK*

BChE activity is slightly lower than normal

Date: *February 28, 2000*

KLINIČKA BOLNICA "MERKUR"
ZAVOD ZA KLINIČKU KEMIJU

PREZIME I IME *D. N.*

KARTICA UPOZORENJA

Osoba je osjetljiva na

SUXAMETHONIUM
i MIVACURIUM

Zavod za kliničku kemiju
Jedinica za fenotipiranje kolinesteraze
Adresa: 10000 Zagreb, Zajčeva 19
Tel.: ..385 1 2431 390 (438)

PREZIME I IME *D. N.*

God. rođ.: *1979.*

Rezultati pretrage Vaše krvi pokazuju da ste osjetljivi na kratkodjelujuće mišićne relaksanse Suxamethonium i Mivacurium. Ovu karticu potrebno je nositi uvijek sa sobom i treba je pokazati liječniku u slučaju da morate primiti anestetik.

FENOTIP: *AK*

Aktivnost BChE je lagano snižena prema normali

Nadnevak: *28.2.2000.*

Fig. 1. Warning Card issued to a patient, who has a suxamethonium sensitive butyrylcholinesterase phenotype.

TABLE 2
DISTRIBUTION OF BCHE PHENOTYPES WITH CORRESPONDING ACTIVITIES AND INHIBITOR NUMBERS IN 7 FAMILIES OF THE PATIENTS* WHO WERE SENSITIVE TO SUXAMETHONIUM.

	Family 1				Family 2				Family 3			Family 4		
	Parent		Child		Parent		Child		Parent		Child	Parent		Child
	M	F	M	F*	M	F	M*	F	M	F	M*	M*	F	M
BChE Activity	7170	4284	7180	7105	6170	1922	2868	7680	6916	5309	6438	6420	5429	8471
DN	82	70	81	70	70	60	22	80	73	81	71	73	82	82
FN	54	59	56	60	60	63	85	52	54	49	55	55	48	51
UN	48	56	43	55	57	58	97	45	53	42	57	55	44	45
RoN	96	81	95	79	82	78	11	97	82	96	79	81	97	96
Phenotype	UU	UA	UU	**UA**	**UA**	**UA**	**AA**	UU	UA	UU	**UA**	**UA**	**UU**	**UU**

	Family 5				Family 6				Family 7				
	Parent		Child		Parent		Child		Parent		Child		
	M (deceased)	F	M	F*	M	F	M	F*	M	F	M	F*	F
BChE Activity		4073	1019	1111	3583	2859	6515	555	6891	6792	8866	3960	4969
DN		81	25	23	80	79	80	89	81	72	82	59	74
FN		53	77	78	52	51	52	47	52	59	54	58	59
UN		47	92	92	45	40	45	44	43	53	46	62	54
RoN		96	20	15	99	97	99	100	96	83	97	66	85
Phenotype		**US**	**AS**	**AS**	**US**	**US**	UU	**US**	**UU/UK**	UA	UU	AK	UA

M – male, F – female
Phenotypes expecting to be sensitive to suxamethonium are in bold letters

Discussion

Although human serum BChE has no known physiological function, it has an important pharmacological property of breaking down the short acting muscle relaxants, suxamethonium (succinyldicholine, scoline) and mivacurium[24]. Detection of BChE variants which lack this property is essential for the identification of patients susceptible to prolonged paralysis in order to counsel them and any close relatives who may be similarly affected.

Summarizing our all results on the Croatian population of 1545 individuals, 93% possessed only the usual enzyme. The frequency for UA, UF, AK, AA, US, AF, FF and AS phenotypes is 4.8, 1.5, 0.32, 0.32, 0.19, 0.13, 0.065 and 0.065, respectively. On average 90% of the European population possess only the usual BChE while 1 in 3500 individuals is homozygous for the atypical variant (AA)[4,7]. On this basis one can predict that about 1300 persons in Croatia are likely to have homozygous atypical BChE. Our value for the atypical variant frequency might be an overestimate because some individuals were included into the cohort studied due to their reaction to suxamethonium rather than being chosen at random. It

has been reported that the atypical variant occurs more commonly in Mediterranean regions than in the northern countries[1,6,7] which might also be an additional reason for somewhat higher frequency of atypical allele in the Croatian population.

In addition to genetic abnormalities, there are other causes of low BChE activities. Exposure to organophosphates or to therapeutic and narcotic drugs like bambuterol and cocaine, disease, especially that involving the liver, and pregnancy, all reduce enzyme activity[1,2,6]. Low BChE activity, either genetically inherited or acquired, is unfavorable for individuals if muscle relaxants like suxamethonium or mivacurium should be administered. Many studies show that an abnormal response to a normal dose of succinylcholine is not always caused by abnormal butyrylcholinesterase genotypes. The abnormal butyrylcholinesterase accounts for abnormal response to succinylcholine only in 60% of cases[11]. At present the screening of patients for low BChE activity prior to surgery is justified if there is evidence of potential risk. BChE analysis using molecular biology at the DNA level can improve biochemical diagnosis[9–11]. DNA analysis provides the precise location of nucleotide alterations in the variants. However, DNA sequencing is time consuming and still not commonly implemented. Biochemical analysis is reliable and provides sufficient information on the BChE status[10]. In circumstances when there are mass casualties information on BChE status might be valuable if facilities for providing artificial ventilation are limited.

Conclusion

The Cholinesterase Unit was established to provide a central service for determining inherited serum butyrylcholinesterase variants sensitive to suxamethonium or mivacurium and to detect individuals showing an abnormal response to the compounds. Once established, the genotype of BChE does not change during the lifetime. Therefore, early detection of this genetic abnormality is very important for patients undergoing a surgical procedure. The carriers of an abnormal phenotype of BChE receive a »Warning Card« as a permanent warning against succinylcholine administration as well as a warning to the members of the their families to test their own phenotype of BChE.

Acknowledgements

Our gratitude is extended to Dr. Nada Jagarinec, who encouraged us to start the project and gave us a valuable help. We thank Dr. R. T. Evans, Leeds, UK, for his stimulating interest and valuable advice concerning the establishment of the Cholinesterase Unit. This study is part of the projects 0044002 and 0022014 supported by the Ministry of Science, Education and Sport of the Republic of Croatia.

REFERENCES

1. ASHANI, Y., Drug Development Research, 50 (2000) 298. — 2. EVANS, R. T., CRC Crit. Rev. Clin. Lab. Sci., 23 (1986) 35. — 3. KUTTY, K. M., Clin. Biochem., 13 (1980) 231. — 4. LOCKRIDGE, O., Pharmac. Ther., 47 (1990) 35. — 5. McQUENN, M. J., Clin. Chim. Acta, 237 (1995) 91. — 6. SCHWARTZ, M., D. GLICK, Y. LOEWENSTEIN, H. SOREQ, Pharmac. Ther., 67 (1995) 283. — 7. WHITTAKER, M., Cholinesterase. In: BECKMAN, L. (Ed.): Monographs in human genetics. (Karger, Basel, 1986). — 8. WHITTAKER, M., Anaesthesia, 35 (1980) 174. — 9. JENSEN, F. S., L. R. NIELSEN, M. SCHWARTZ, Human Heredity, 46 (1996) 26. — 10. CERF, C., M. MESQUISH, I. GABRIEL, S. AMSELEM, P. DUVALDESTIN, Anesth. Analg., 94 (2002) 461. — 11. JENSEN, S., J. VIBY-MOGENSEN, Acta Anesth. Scand., 39 (1995) 150. — 12. FLEGAR-MEŠTRIĆ, Z., B. ŠURINA, Z. ŠIFTAR, Chemico-Biological Interactions, 119—120 (1999) 193. — 13.

SIMEON, V., A. BUNTIĆ, B. ŠURINA, Z. FLEGAR-MEŠTRIĆ, Acta Pharm. Jugosl., 37 (1987) 107. — 14. SIMEON-RUDOLF, V., R. T. EVANS, Arh. Hig. Rada Toksikol., 52 (2001) 299. — 15. SIMEON-RUDOLF, V., R. T. EVANS, Acta Pharm., 51 (2001) 289. — 16. DAS, P. K., J. LIDDELL, J. Med. Genet., 7 (1970) 351. — 17. KALOW, W., K. GENEST, Can. J. Biochem., 35 (1957) 339. — 18. HARRIS, H., M. WHITTAKER, Nature, 191 (1961) 496. — 19. HANEL, H. K., J. VIBY-MOGENSEN, Br. J. Anaesth., 49 (1977) 1251. — 20. EVANS, R. T., J. WARDELL, J. Med. Genet., 21 (1984) 99. — 21. TURNER, J. M., R. A. HALL, M. WHITTAKER, R. L. HOLDER, L. J. KRICKA, Ann. Clin. Biochem., 22 (1985) 175. — 22. ŠIFTAR, Z., B. ŠURINA, Biochemia Medica, 5 (1995) 23. — 23. REINER, E., V. SIMEON-RUDOLF, M. ŠKRINJARIĆ-ŠPOLJAR, Toxicology Letters, 82/83 (1995) 447. — 24. FRAMPTON, J. E., D. McTAVISH, Drugs, 45 (1993) 1066.

B. Šurina

Institute of Clinical Chemistry, Clinical Hospital »Merkur«, Zajčeva 19, 10000 Zagreb, Croatia

UVOĐENJE »KARTICA UPOZORENJA« ZA NOSIOCE ABNORMALNIH BChE VARIJANTI

S A Ž E T A K

Prepoznavanje varijanti butirilkolinesteraze (E.C. 3.1.1.8) u ljudskom serumu posebno je važno kako bi se identificirao pacijent koji je osjetljiv na produljeno djelovanje kratkodjelujućih mišićnih relaksansa, poput sukcinilkolina (suxamethonium, scolinehidroklorid) i mivakurija. Neke varijante butirilkolinesteraze veoma sporo hidroliziraju sukcinilkolin i prilikom njihove aplikacije može nastupiti produžena paraliza mišića. Ako se na vrijeme otkriju te nasljedne varijante u serumu, pacijent i njegovi rođaci, koji mogu biti slično osjetljivi, dobiti će odgovarajući stručni savjet i izbjeći moguće komplikacije. Stoga je u Zavodu za kliničku kemiju Kliničke bolnice Merkur osnovana Jedinica za fenotipiranje butirilkolinesteraze s podacima o obiteljima kod kojih su dokazane urođene varijante serumske butirilkolinesteraze osjetljive na suxametonij. U početnom istraživanju određene su varijante i fenotip butirilkolinesteraze u serumima 384 pacijenata i 7 njihovih obitelji. Ustanovljene su »Kartice upozorenja« kao informacija od važne medicinske naravi, a izdane su dvojici pacijenata s abnormalnim BChE varijantama, da bi se upozorilo anesteziologa na mogućnost ekspresije prolongirane apnee prilikom primjene kratkodjelujućih mišićnih relaksansa.