AMPATHCHAT no. 87, version 2

DEMYSTIFYING CHOLINESTERASE TESTING: OUR INNOVATIVE IN-HOUSE SOLUTION TO BIOMONITORING

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KEY MESSAGES

- Organophosphates and carbamates are classified as cholinesterase inhibitors.
- Monitoring cholinesterase levels in exposed workers is a legal requirement.
- Whole-blood cholinesterase is the superior marker of cholinesterase inhibition when compared to plasma cholinesterase.
- Plasma cholinesterase levels are not specific enough for low-grade organophosphate exposure and are influenced by a wide variety of factors. These levels are more useful in the emergency setting with acute organophosphate poisoning.

BACKGROUND

The monitoring of cholinesterase levels plays a fundamental role in assessing exposure to organophosphates and carbamate pesticides. A recent decline in the use of these conventional pesticides is seen in developed countries and in countries that are members of the European Union, especially due to their bio-accumulation potential and environmental risks.[1]

However, the agricultural sectors in most developing countries still use the conventional organophosphate- and carbamate-containing pesticides on a large scale – mainly due to affordability, effectivity and availability, and therefore still necessitates the accurate biomonitoring of exposed workers.[2]

Cholinesterase levels are useful in identifying workers that are **overly** exposed to pesticides that inhibit this important enzyme. Suppression of this enzyme dictates intervention in the form of medical treatment, alternative work placement or step-up in the use of personal protective equipment, etc.

The biological monitoring of workers exposed to organophosphate or carbamate is a legal requirement in South Africa, in terms of the *"Regulations for Hazardous Chemical Agents 2021"* under section 43 of the Occupational Health and Safety Act (Act No. 85 of 1993).

CHOLINESTERASE SUBTYPES

	Acetylcholinesterase	Butyrylcholinesterase
Synonym	• True cholinesterase • RBC cholinesterase • Red-cell cholinesterase	• Pseudocholinesterase • Plasma cholinesterase
Location	• CNS • NM-junctions • RBC membranes • Autonomic nervous system	• Synthesis in liver • Plasma • Low concentration in the white matter of the CNS, e.g. hippocampus, amygdala[16]
Half-life	Similar to that of an RBC; ± 120 days[14]	Shorter half-life; <10 days
Functions	Hydrolyses the neurotransmitter acetylcholine into acetic acid and choline[13]	Hydrolyses of choline and non-choline esters, e.g. succinylcholine and mivacurium

RBC = Red blood cell; CNS = Central nervous system; NM = Neuromuscular

PATHOPHYSIOLOGY OF CHOLINESTERASE INHIBITION

Acetylcholine —(cholinesterase)→ **choline + acetate**

Cholinesterase is an enzyme that mainly functions as part of the nervous system, where it catalyses the hydrolytic breakdown of acetylcholine (ACh) and thereby terminates neurotransmission. Cholinesterases are found in the central and peripheral nervous systems, as well as in muscles at the neuromuscular junction (NMJ).

Cholinesterase inhibitors (i.e. organophosphates or carbamates) are structurally similar to acetylcholine and can bind to both subtypes of cholinesterase enzymes; organophosphates will phosphorylate and the carbamates will acetylate a serine residue of the enzyme's active sites, thereby rendering the enzyme irreversibly inactive.[4]

Subsequently, ACh accumulates at the neuromuscular junction causing continuous stimulation of the post-synaptic neuron receptors. This explains the classic fasciculation, paralysis or respiratory failure seen with acute organophosphate poisoning.[5,6]

Chronic low-grade cholinesterase inhibition, on the other hand, produces an insidious clinical picture with lower neonatal birthweights[8], neuro-psychological impairment[7] and chronic lung disease, e.g. asthma and chronic obstructive lung disease.[9]

CHOLINESTERASE LEVELS IN BIOLOGICAL MONITORING

Cholinesterase has a wide population reference interval. It is advisable for a patient result to be interpreted in relation to a personal reference value – dictating that at least one baseline cholinesterase level be done prior to cholinesterase inhibitor exposure. This becomes crucial in low-level exposures due to the smaller changes in enzyme concentration that could potentially be missed and result in chronic disease.[11]

Discretionary pre-shift/post exposure (generally baseline taken during season or peak application period) recommended. A true Baseline Level is taken 4 weeks after non-exposure; ideally 2 baseline measurements are to be done 3–14 days apart and should agree to 15–20%. A reduction of 30% or more from a basal (pre-exposure) level may indicate organophosphate or cholinesterase inhibitor toxicity/exposure.

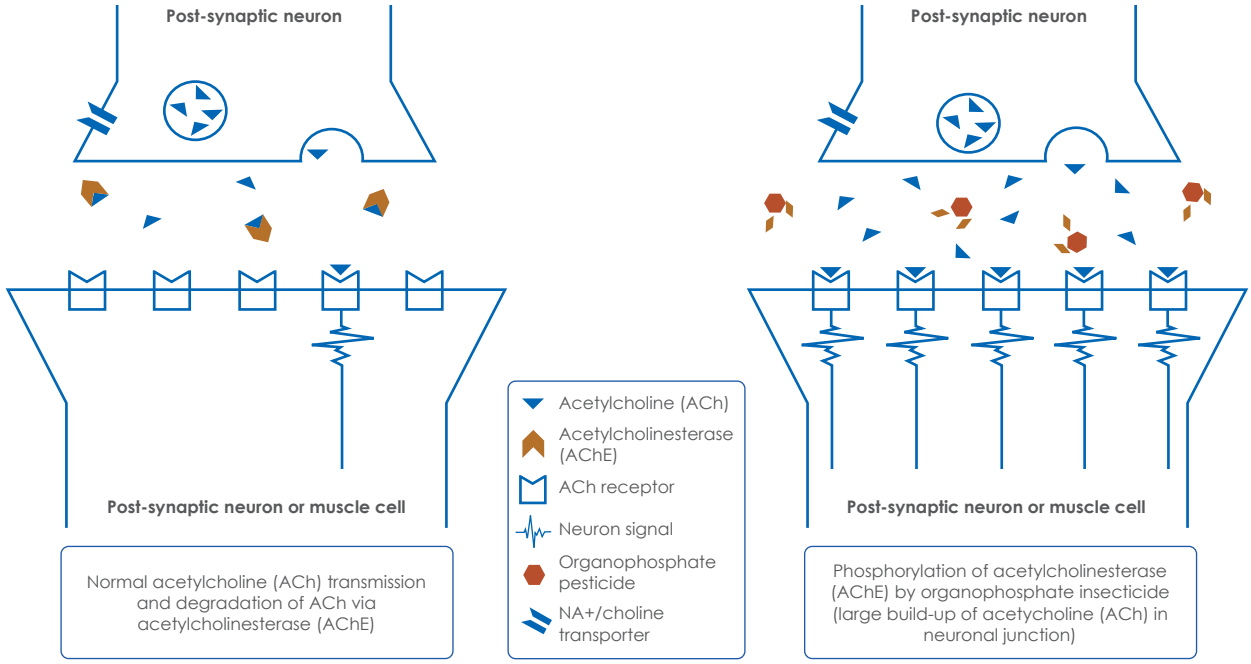

FIGURE 1: THE PHYSIOLOGICAL ACTION OF ACETYLCHOLINE AT THE NEURONAL CELL SYNAPSE, THE BREAKDOWN OF ACh THROUGH ACETYLCHOLINESTERASE AND THE PHOSPHORYLATION OF AChE THROUGH ORGANOPHOSPHATE[10]

WHAT ENZYME IS THE MOST SUITABLE FOR THE BIOMONITORING OF CHOLINESTERASE INHIBITION?

Whole-blood cholinesterase =
RBC cholinesterase + plasma cholinesterase

Whole-blood cholinesterase

From a practical perspective and due to speed of analysis, simplicity and cost, the determination of cholinesterase activity in a whole-blood sample is used quite often in the monitoring of exposure to cholinesterase inhibitor compounds.[12] This measurement gives an indication of both the red blood cell cholinesterase and plasma cholinesterase enzyme activity.

Red blood cell cholinesterase: hydrolyses acetylcholine

Red blood cell cholinesterase is also referred to as true cholinesterase or acetylcholinesterase (AChE) and is the primary cholinesterase subtype found at the synapses in the nervous system, skeletal muscle, placenta and, of course, the red blood cell membranes.

The AChE activity in erythrocytes is possibly the most suitable due to its analytical sensitivity, precision and specificity, being the Ellman's spectrophtometric method, the most internationally recommended method for analysis.

This enzyme is measured in the detection of chronic or previous exposure to cholinesterase inhibitors. It correlates best with the cholinesterase concentration in the nervous system. It is considered the best indicator of cholinesterase inhibition in the nervous system because of the longer half-life of >120 days – similar to that of the lifespan of a red blood cell.[14]

This test is no longer offered by Ampath and has been replaced with the whole-blood cholinesterase assay.

Plasma cholinesterase: hydrolyses butyrylcholine
Plasma cholinesterase is also referred to as pseudocholinesterase or butyrylcholinesterase (BChE) and is more widely distributed in the body, being the predominant subtype found in the liver, brain, kidney, intestine, pancreas and, of course, plasma.

A correlation between the suppression of BChE-levels and the severity of acute organophosphate poisoning exists and further correlates with patient outcomes in the acute setting. It is still indicated in the acute setting by most emergency medicine guidelines, although levels do not always give the best indication of the degree of poisoning.[13] Levels are influenced by a variety of conditions (e.g. liver disease, malnutrition and pregnancy) and the short half-life of about eight days. This makes this a less than ideal indicator of chronic exposure.[12]

TESTING WHOLE-BLOOD CHOLINESTERASE

The test is performed on whole blood and is collected in a tube containing EDTA. The specimens are manually haemolysed. This enables the red blood cells to burst open and free the AChE to make it available for testing. The enzyme activity is measured by means of a colorimetric reaction.

This test is currently offered by Ampath's Specialised Chemistry Department in the National Reference Laboratory (NRL).

For more information or assistance, contact your local Ampath representative.

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PUBLISHED: MAY 2024