**Multifunctional neuron-specific enolase: its role in lung diseases**

Cai-Ming Xu1,2,* , Ya-Lan Luo1,2,* , Shuai Li3, Zhao-Xia Li4, Liu Jiang1, Gui-Xin Zhang1, Lawrence Owusu1,4 and Hai-Long Chen1

1Department of General Surgery, The First Affiliated Hospital, Dalian Medical University, Dalian 116011, Liaoning, China; Institute (College) of Integrative Medicine, Dalian Medical University, Dalian 116044, Liaoning, China; 2Department of Traditional Chinese Medicine, Dalian Obstetrics and Gynecology Hospital, Dalian 116021, Liaoning, China; 3Department of Pediatric Orthopedics, Qingdao Women and Children’s Hospital, Qingdao 266035, Shandong, China; 4Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), PMB, UPO, Kumasi, Ghana

Correspondence: Hai-long Chen (chenhailong2007@yahoo.com) or Lawrence Owusu (dady_k_jrn@yahoo.com)

Neuron-specific enolase (NSE), also known as gamma (γ) enolase or enolase-2 (Eno2), is a form of glycolytic enolase isozyme and is considered a multifunctional protein. NSE is mainly expressed in the cytoplasm of neurons and neuroendocrine cells, especially in those of the amine precursor uptake and decarboxylation (APUD) lineage such as pituitary, thyroid, pancreas, intestine and lung. In addition to its well-established glycolysis function in the cytoplasm, changes in cell localization and differential expression of NSE are also associated with several pathologies such as infection, inflammation, autoimmune diseases and cancer. This article mainly discusses the role and diagnostic potential of NSE in some lung diseases.

**Introduction**

**Enolase**

Enolase (EC 4.2.1.11) is a well-known glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate into phosphoenolpyruvate and its reverse reaction during gluconeogenesis. Enolase is involved in the glycolytic pathway, which decomposes glucose into pyruvate and produces both high-energy compounds of ATP and cofactor NADH, providing energy and material basis for cell metabolism and various life activities (Figure 1). Therefore, enolases are among the most ubiquitously and abundantly expressed proteins in cells from archaeabacteria to mammals, with highly conserved amino acid sequence [1,2]. Many studies have suggested that enolase, as well as pyruvate kinase and hexokinase, may dominate in metabolic contributions to inflammation and facilitate tumour proliferation under hypoxic conditions by elevating glycolysis [3,4].

Functionally, active enolases are dimers. They consist of non-covalently linked dimers of either α, β or γ subunits, which are expressed by different genes. These genes are not considered housekeeping genes because their expression is tissue-specific and varies during developmental, metabolic or pathophysiological conditions [5]. Besides the peptide molecules, enolases also require divalent metal ions to maintain their structural stability and catalytic activity, especially Mg2+ [6] (Figure 2). There are mainly three isozymes of enolase in vertebrate organisms: α-enolase (Eno1), β-enolase and γ-enolase, which are homodimers composed of two of the same subunits [1]. β and γ subunits readily form mixed dimers with the α subunit; the intermediate form is a hybrid molecule containing α and β/γ subunits. All possible dimers except βγ have been observed in vivo. The three enolase isoforms share high-sequence identity and kinetic properties [7–10]. Each subunit of enolase is made up of two domains: the smaller N-terminal extending from amino acid 1 to approximately 134 and the larger C-terminal domain (residues from approximately 143 to 434). The N-terminal domain has a βα barrel structure with βαζ(βαζ)6 topology [10]. Between these domains is a short random structure fragment. This characteristic short variable region is situated predominantly on the surface of...
Figure 1. Enolase is a well-known glycolytic enzyme that catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate during glycolysis and gluconeogenesis

Enolase, in the glycolytic pathway, is involved in the breakdown of glucose into two pyruvate molecules and the high-energy compounds ATP and cofactor NADH, providing energy and material basis for cell metabolism and various life activities. Under normoxic conditions (left panel), most of the cellular energy is obtained from oxidative phosphorylation in the mitochondria with O₂ as the terminal electron receiver in the electron transport chain to generate ~34 ATPs/glucose molecule. However, in the absence of O₂ or under reduced oxygen conditions (hypoxia, right panel), cells resort to substrate level phosphorylation by increasing the rate of glycolysis to generate ATPs from the process. The expressions and activities of glycolytic enzymes hexokinase, enolase and pyruvate kinase are elevated to facilitate the process. Cell damaging and cancer promoting factors, including ROS, are by-products of such inefficient cellular respiratory process.

Figure 2. A simple analysis of space conformation of NSE: NSE is a metal-activated enzyme composed of two asymmetric γ subunits (brown and grey)

Two types of Mg²⁺ binding sites contribute to catalysis. Mg²⁺ binding in site I (yellow above), traditionally called ‘conformational’, induces a conformational change in the enzyme and enables binding of substrate or substrate analogues. The second Mg²⁺ ion (yellow below), called ‘catalytic’, can bind in site II and then the catalytic reaction occurs. The second Mg²⁺ ion binds in the catalytic metal ion binding site in the closed conformation loop 36–43, which includes a residue crucial for catalysis: Ser³⁹ (red). γ-enolase crystal structure (1TE6) was obtained from Protein Data Bank (PDB). The image was prepared by authors with Python.
the molecule, far away from the active site. Additionally, residues that participate in catalytic activity are to a large extent conserved throughout [10,11]. The conservation of flanking residues on either side of these catalytic residues also indicates that the basic folding structure of all enolases is essentially the same [10]. α-Enolase exists in all fetal and most adult mammal tissues. However, it is replaced by other isoforms during tissue development [1]. The α-enolase levels in embryonic brain are high and decrease with the maturation of neurons. Conversely, γ-enolase levels are considered to be low in embryonic brain, and increase with the development of nervous system structure and function, indicating that there are two isoenzymes of enolase in mature brain: γ-enolase is proved to restrict to neuronal cells, hence the traditional name, neuron-specific enolase (NSE), while α-enolase is limited to glial cells (non-neuronal enolase, NNE) [12]. α-Enolase can be replaced by γ-enolase in the brain, which is a late event in the development of the nervous system and can be a marker of neural maturity [13,14]. Similarly, during ontogenesis, α-enolase is replaced by β-enolase in the muscle group [5]. The most significant difference between α-enolase (NNE) and γ-enolase (NSE) is the obvious lack of immunological cross-reactivity between the two proteins [15,16]. NNE is highly sensitive to chloride ions, urea and temperature. In contrast, NSE is significantly more stable against chloride-induced inactivation. NSE’s relative insensitivity to chloride ions is particularly interesting since chloride ions accumulate in nerve cells during repeated depolarization. The relative resistance of NSE to chloride ions may have evolved to adapt to the intracellular environment of neurons, thus preventing inactivation of chlorine-sensitive enolase in neurons when neurons depolarize, and interrupting glycolysis when metabolic energy is most needed [17]. Additionally, NSE has been proposed to exhibit higher or enhanced catalytic rate as such their preference by cancer cells and in obesity where there is increased energy demand, usually under reduced oxygen conditions [18,19]. Following the initial discovery and classification of γ-enolase as neuron specific, its presence in other non-neural cell/tissue types such as fibromuscular tissue of the prostate, lymphocytes, the myometrium of the uterus, spermatogonia, myoepithelial cells, the heart, and macula densa cells of the kidney, megakaryocytes and platelets has been empirically demonstrated [20]. Enolases are predominantly located in the cytoplasm of cells and exert catalytic effects. However, recent accumulation of evidence has revealed that they can also be detected in the nucleus, cell membrane and extracellular space, showing some other functions. For instance, α-enolase has been shown to act as a plasminogen receptor on the surface of several eukaryotic cells (such as neurons [21], monocytes [22], B cells, T cells and endothelial cells [23]), binding to plasminogen and protecting it from its inhibitor α-antiplasmin. The bound plasminogen is cleaved by specific protease to generate active plasmin, which degrades ECM, facilitating pathogen invasion, infiltration of inflammatory cells and migration of tumours [24] to participate in various pathophysiological processes including inflammation, myogenesis [25,26], tumorigenesis and angiogenesis [27]. α-Enolase also exhibited strong plasminogen binding activity on the surface of the prokaryote Streptococcus pneumoniae and contributes to its virulence potential during invasive infection [11]. Feo et al. [28] and Subramanian et al. [29] also observed α-enolase in the nucleus, playing an important role in the form of an alternative translation product as c-myc promoter-binding protein-1 (MBP-1). MBP-1 binds c-myc P2 promoter and negatively regulates transcription of the protooncogene. Furthermore, α-enolase has also been described as a heat-shock protein [30] and a hypoxic stress protein [31].

β-Enolase may segregate to different subcellular sites where they can respond to specific functional demands. In muscle cells, the structural features of the β-enolase allow it to localize in the naked areas of actin thin filaments (the I band of the sarcomere) to furnish the ATP for muscle contraction along with other glycolytic enzymes [32].

NSE

γ-Enolase, also known as NSE, is a soluble cerebral protein, first described by Moore and McGregor in 1965 [33]. It is mainly located in the cytoplasm of central and peripheral neurons and neuroendocrine cells [34]. Small amounts of NSE were also found in non-neuronal and non-neuroendocrine cells or tissues, such as platelets, erythrocytes, prostate, breast tissue and uterus [20,35]. The organ localization of NSE results in the elevation of its serotype associated with various nerve damages such as ischaemic stroke, intracerebral haemorrhage, post-traumatic brain injury and spinal cord injury. Thus, the level of NSE measured in blood and cerebrospinal fluid may be a potentially useful marker for assessing neuronal death in different centre neuronal system injuries, with increased correlation to injury severity [36]. Expression of cell surface γ-enolase initiates inflammatory reaction after injury. Following injury, NSE rapidly transfer from the cytosol of cells (e.g., neurons, glial cells and inflammatory cells) to the surface. On the one hand, it enhances cell antigen presentation and degrades ECM through concentrating plasminogen on the surface; on the other hand, NSE could drive the production of ROS, NO and various cytokines (such as TNF-α, IL-1β, INF-γ, TGF-β and MCP-1) via pro-inflammatory signaling pathway to bolster inflammation [27,37–39]. Additionally, NSE can play a protective role in promoting cell survival and act as neurotrophin in neurons. Its hydrophobic domain in the N-terminal region enables it to dock on the surface of plasma membrane of neurons, glia and astrocytic cells.
The C-terminal part of the molecule could promote neuronal survival, differentiation and axonal regeneration by activating the signalling pathways of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). NSE-mediated PI3K activation also regulates RhoA kinase [40], which can influence actin cytoskeleton reorganization, induction of neurite outgrowth, and growth arrest in neuronal cells, for negative regulation [27,37]. This additional function could be important also in cancer cells (as discussed later). Several studies have confirmed the ability of a synthetic peptide of 30 amino acids mimicking the C-terminal part of NSE to promote survival of rat neocortical cells in vitro [41]. While cathepsin X can abolish this neurotrophic activity by cleaving the C-terminal of NSE, γ-1- syntrophin enhances this neurotrophic activity by trafficking NSE towards the plasma membrane [42,43]. In addition, Hafner et al. [44] observed large amount of C-terminally cleaved form of γ-enolase around the lesion (amyloid plaque) in the brain of transgenic mice of Alzheimer’s disease while its intact form mainly existed in microglia cells near to senile plaque. It was confirmed in mouse microglial cell line that amyloid-β peptide can induce up-regulation of γ-enolase, which plays a protective role in amyloid-β-related neurotoxicity. Furthermore, Pislar et al. [45] also found that the C-terminal of NSE can impair amyloid-β-induced apoptosis by interacting with p57 neurotrophin receptor (p57NTR) to inhibit the activation of its downstream signalling factors. These experimental results showed that although γ-enolase on the cell surface is not like α-enolase which have C-terminal lysine binding to plasminogen [46], its C-terminal specific amino acid sequence can play dual role in the pathophysiological process of the nervous system through multiple signal pathways [37,42,43,47]. In addition to membrane localization of γ-enolase, studies from Soh have also observed a significant nuclear localization of NSE in Cd²⁺- and As³⁻- transformed breast epithelial and urothelial cell lines. When NSE expression in a given cell is low, it tends to be localized in the nucleus, while when NSE expression is high, it is localized in both the nucleus and cytoplasm. The discovery of the nuclear localization indicates that enolase functions extend far beyond glycolytic activity [35,48].

γ-Enolase might also have a multifunctional role in cancer progression. As a cytoplasmic enzyme, it provides energy support for the survival and unlimited proliferation of cancer cells through increased aerobic glycolysis (Warburg effect, Figure 1) [49–51]. Moreover, Vizin and Kos [52] have revealed a new mechanism of γ-enolase involvement in cancer progression: upon stimulation, the cellular localization of γ-enolase changes, and can traffic to the cell surface to activate survival promoting signaling pathways as it does in neuronal cells and to promote migration of tumour cells. This in effect helps tumour cells adapt to stressful conditions (hypoxia, chemotherapy, radiotherapy and serum starvation). For instance, existing experiments have found that when glioblastoma cells were in a state of serum starvation and hypoxia, the expression of γ-enolase in the cells was significantly increased, and the expression level of survival promoting proteins as well as anti-apoptosis signal pathways (which are triggered by γ-enolase as in neurons) were significantly elevated [53–55]. In addition, Yan et al. [53] observed that knocking-out γ-enolase significantly reduced migration of tumour cells. It may be related to the function of γ-enolase in promoting dynamic remodelling of actin cytoskeleton, which is an important prerequisite for cell migration [56]. The precise mechanism by which NSE induce actin remodelig is currently not very clear. The author regarded that γ-enolase may induce actin polymerization and redistribution by interacting with actin filaments via γ-1-syntrophin and regulating RhoA kinase (A regulator of actin cytoskeleton organization) [37,43,57]. γ-Enolase is usually overexpressed in tumours of neurogenic and neuroendocrine origin and is used as a marker for detecting neuroendocrine differentiation of tumour cells. At present, many studies have shown that γ-enolase is of certain significance in the diagnosis, treatment monitoring and prognosis of various neuroendocrine tumours, especially lung cancer.

NSE assays in tissues and body fluids
Assays for NSE concentration and activity determinations have seen continuous innovation with the progress of science and technology. Several qualitative or quantitative techniques have been developed to detect NSE in tissues and body fluids (serum, cerebrospinal fluid, urine etc.).

Radioimmunoassay
As early as the 1970s, a solid-phase radioimmunoassay (RIA) using ³H-labelled antigen and double antibody to determine NSE and NNE in brain tissue was described by Marangos et al. [13,58,59]. It was also widely used for the determination of NSE in other tissues, but it was not sensitive enough to measure NSE of nanogram amount in body fluids such as cerebrospinal fluid and cell culture fluid [58,59]. Parma et al. [60] proposed an improvement by labelling antigen with [125] I. Because the activity of NSE labelled with [125] I was higher than that with ³H, the improved sensitivity allowed the determination of low amounts of proteins in body fluids. In 1989, Paus and Nustad [61] applied monoclonal antibody against NSE and mono-disperse magnetizable particles (as solid phase carriers) to immunoradiometric assay (IRMA). The measurement range of this test was 0.4–170 µg/l. Compared with RIA using polyclonal
antibodies, the incubation time was shorter, with higher sensitivity and accuracy [62]. RIA is a traditional method for detecting tumour markers. It has the advantages of high sensitivity, easy commercialization and so on. However, compared with other methods, it has the disadvantages of short service life of kits, radioactive pollution risks and so on. At present, it has gradually been replaced by other detection methods.

**Enzyme immunoassay**

In 1987, Anastasiades et al. [63] developed an enzyme-linked immunosorbent assay (ELISA) using double antibody sandwich method [64] to measure NSE concentration in serum of patients with small cell lung cancer (SCLC). The sandwich used in this system was rabbit anti-rat enolase that cross-reacts with the human γ monomer, resulting in the test being specific for only the γγ isoenzyme. An avidin–biotin–peroxidase complex system was used to provide increased assay sensitivity. Following Schmitt’s two-step enzyme immunoassay (EIA) using a specific monoclonal antibody against NSE in conjunction with a polyclonal antibody to measure NSE level in serum [65], Ebert et al. proposed a new detection method at the beginning of the 20th century: the New Elecsys NSE assay, which is a single-step, solid-phase EIA of a sandwich type employing two specific monoclonal antibodies raised in mice against purified γ-enolase from human brain [66]. They assessed its utility as a sensitive and specific test for the diagnosis of SCLC and hold the view that the Elecsys NSE ELA is a reliable and accurate diagnostic procedure to measure NSE of serum samples with a wide measuring range (up to 370 ng/ml) and a short incubation time (18 min).

**Immunobioluminescent assay**

In 1983, Wevers et al. [67] determined NSE in human serum and cerebrospinal fluid by bioluminescent assay using luciferine–luciferase system. A solid-phase immune-bioluminescence assay for NSE in human plasma was also developed by Gerbitz et al. [68] in 1984. Purified antibodies against NSE and NNE are coated on to polystyrene tubes. Each sample containing the isoenzyme activity to be determined is incubated in the respective coated tube. The coating is then washed and the reaction of the antibody-bound enzyme is initiated by adding 2-phosphoglycerate, ADP and pyruvate kinase. ATP formed in the enzymatic reaction is measured by following the increase in light emission in the firefly luciferase bioluminescent system. In 1986, Viallard and colleagues [69,70] proposed two new methods using bioluminescence assay for the determination of NSE in serum. The key steps for these two assays were immunocapture of enolase isoenzymes containing γ subunit and electrophoretic separation of the isoenzymes on cellulose acetate respectively. At present, adenosine triphosphate bioluminescent assay is widely used in microbial biometrics. Its role in tumour marker detection is gradually replaced by chemiluminescent immunoassay.

**Chemiluminescence analysis**

Chemiluminescence immunoassay uses chemiluminescent substances (e.g., luminol) or enzymes (e.g., alkaline phosphatase, ALP) as labels to directly label antigens or antibodies, forming immune complexes through antigen–antibody reaction. An oxidant or enzyme luminescent substrate is added to form an intermediate in an excited state which could emit photons to return to a stable ground state. The intensity of luminescent signals is proportional to the amount of substances being measured. Fu et al. [71] developed a chemiluminescence EIA based on magnetic nanoparticles (MPs-CLEIA) in 2012 to detect NSE in SCLC patients’ serum. The method connected fluorescein isothiocyanate (FITC) labelled NSE capture antibody and NSE with ALP labelled detection antibody in a sandwich-type manner. This immune complex reacted with anti-FITC coated magnetic beads. Due to the enrichment effect of magnetic field, the method showed high sensitivity (LOD < 0.2 ng/ml) and excellent accuracy, stability (CVs < 10%), and specificity. Compared with the traditional CLEIA method, MPs-CLEIA method was superior. As a result, the MPs-CLEIA method is expected to become a rapid, sensitive and reliable diagnostic for SCLC in large-scale screening due to its advantages of low cost and easy automation.

**Immunosensor**

Natural enzymes that label antigens/antibodies have the disadvantages of poor stability, limited sources, sensitivity to environmental changes, susceptibility to environmental influences and denaturation, and the labelling process usually damages the biological activity of antibody molecules. Therefore, enzyme-free immune systems based on metals and metal complexes, magnetic nanoparticles and quantum dots are continuously being developed. Immunosensors have always been concerned and favoured by tumour researchers. The biosensor combines specific immune reaction with biosensing technology, and the biological recognition part of the biosensor comes from the specific recognition and binding effect of antigen and antibody. Biological signals are converted into electrical signals for detection.
through a physicochemical transducer and a signal amplification device. Metal nanomaterials are often used to construct immunosensors due to their unique optical, electronic and catalytic properties. Ho et al. [72] developed an electrochemical sandwich immunosensor. This Au nanoparticle congregate-based assay provides an amplification approach for detecting Enol1 at trace levels, leading to a detection limit as low as 11.9 fg. In addition, carbon nanomaterials and polymer composites are also widely used in the manufacture of immunosensors due to their good mechanical properties and redox properties. In 2013, Li and Tian [73] developed a new and enzyme-free electrochemical immunoassay protocol for the sensitive electronic monitoring of NSE on a monoclonal mouse anti-human NSE antibody (mAb)-modified glassy carbon electrode, using guanine-decorated graphene nanostructures (GGNs) as nanotags. Recently, Wei et al. [74] used an Au nanoparticle/reduced graphene oxide composite (AuNP-RGO), a signal-enhanced electrochemical immunosensor without label to detect NSE. In addition to the above materials, other materials with different functions are often introduced to improve the performance of immunosensors. For instance, quantum dots [75] are used to improve sensor sensitivity for their high surface activity, small size, and sensitivity to light, electricity and temperature. Sensors constructed with mesoporous material with good pore structure and interface structure [76] can maintain good activity and functionality of the enzyme. Sensors consisting of hydrogel [77] have good stability and high solubility, and can respond to external stimuli to conduct corresponding changes.

**Proteomics**

Recently, Zhong et al. [78] developed an on-chip spyhole nanoelectrospray ionization mass spectrometry for sensitive biomarker detection in small volumes. This electrospray ionization mass spectrometry uses electrospray to liquidize polypeptides for identifying proteins. This method can ensure the integrity of the sample molecules during ionization and will not fragment ions. Protein chip is a newly developed analysis technology in the past decade. Protein probes are arranged on the surface of supports to capture target proteins, and then qualitative or quantitative analysis is carried out by detectors. Clinically, it is often used to screen and find tumour markers. Liu et al. [79] designed a bead-based microarray chip using a single layer of polydimethylsiloxane (PDMS) as carriers. The assay could be used for simultaneously detecting three lung cancer biomarkers: carcinoembryonic antigen (CEA), fragments of cytokeratin 19 (CYFRA21-1) and NSE in 10 μl of human serum, with a wide linear dynamic range (1.03–111 ng/ml for CEA and CYFRA21-1; 9.26–1000 ng/ml for NSE) and a low detection limit (CEA: 0.19 ng/ml; CYFRA21-1: 0.97 ng/ml; NSE: 0.37 ng/ml; S/N = 3). Furthermore, the current approach is easy to operate without extra driving equipment such as pumps, can make parallel detection for multiplexing with rapid binding kinetics, small reagent consumption and low cost.

**γ-Enolase in lung cancer**

γ-Enolase is expressed only in specific tissues under physiological conditions. Overexpression of NSE and increased level in serum may be related to the malignant proliferation of these tissues, and therefore could be of value in diagnosis, staging, treatment and prognosis of such cancers, especially lung cancer.

According to a systematic analysis from the Global Burden of Disease Study, the number of cases of trachea, bronchus and lung cancer increased by 28% between 2006 and 2016, 2 million people suffering in 2016, and resulting in 1.7 million deaths, making it the most common and lethal human cancer in the world [80]. Early stage diagnosis of lung cancer is of great significance in clinical work. Primary bronchogenic carcinoma (lung cancer) is generally classified into four major cell types by histology: squamous cell lung cancer (SQC), lung adenocarcinoma, large cell lung cancer and SCLC. The first three types are grouped together as non-SCLC (NSCLC) [81]. The differences in behaviour, pathological and clinical characteristics between SCLC and NSCLC contribute to their diagnosis, treatment and prognosis. In addition to histology, an alternative diagnostic method also may be critical, especially if it is based on simple laboratory tests, performed on serum or other body fluid samples.

**SCLC**

SCLC is a subtype of lung cancer with extremely high malignancy, accounting for approximately 15–20% of lung cancer [82]. It often occurs in the middle and old age, with more men than women, and is characterized by its early metastasis, rapid growth and sensitivity to initial chemotherapy [82]. Besides the TNM staging of tumour [83], the ‘two-stage’ method is usually used in clinical research and practice of SCLC. In 1973, VALG proposed two clinical stages of SCLC: limited-stage disease (LD) and extensive-stage disease (ED) [40]. In LD, the lesion is limited to one side of thoracic cavity and can be included in a radiotherapy field. In ED, the tumour has metastasized to the contralateral chest and distant sites. Most of the cases diagnosed are ED at first visit, while LD cases are relatively few (15–25%). SCLC is an aggressive neuroendocrine tumour; therefore, neuroendocrine markers such as NSE, chromogranin A
(CGA) and pro-gastrin-releasing peptide (ProGRP) have been shown to be important to immunohistochemically characterize these malignant lung cancer and can be used as tumour markers released into the circulation [84].

**Diagnosis**

NSE is currently recognized as the most reliable tumour marker in the diagnosis of SCLC. Its serum level differed significantly according to tumour size, disease stage and distant metastasis (all \( P < 0.05 \)), while no association was found with gender or age (both \( P > 0.05 \)) [85]. Kostovski et al. [86] performed a basic immunohistochemical study of four antigenic phenotypes (TTF1, CK7, CK20 and NSE) in 21 patients with lung carcinomas and observed that NSE(+) was found in all cases of SCLC (100%). Another group of experiments reported similar results: the expression of \( \gamma \)-enolase in tumour tissues of SCLC patients was significantly increased, 35-times higher than that of normal tissues, and the \( \gamma/\alpha+\gamma \) value in lung tissues of SCLC patients was significantly higher than that of normal tissues (\( P < 0.01 \)) [87]. In addition, NSE levels in serum are also significantly different between patients and non-patients: increased level of serum NSE (> 13 ng/ml) was detected in 68% of patients with limited SCLC and 87% of patients with extensive SCLC [88]. Huang et al. [89] conducted a meta-analysis of serum NSE levels to establish an evidence-based perspective on its clinical value for screening patients with SCLC. Pooled sensitivity of NSE for detecting SCLC was 0.688 (95% CI: 0.627–0.743), specificity was 0.921 (95% CI: 0.890–0.944), positive likelihood ratio was 8.744 (95% CI: 6.308–12.121), negative likelihood ratio was 0.339 (95% CI: 0.283–0.405). The diagnostic performance was better in Europe than in Asia [sensitivity: 0.740 (95% CI: 0.676–0.795) vs. 0.590 (95% CI: 0.496–0.678), specificity: 0.932 (95% CI: 0.904–0.953) vs. 0.891 (95% CI: 0.819–0.948)]. ELISAs had the highest sensitivity and RIA had the highest specificity. Therefore, serum NSE has high diagnostic efficiency in the early detection of SCLC, but its curative effect varies according to different research region and assay methods. Some studies suggested that tumour markers in exhaled breath condensate (EBC) may have better diagnostic performance for lung cancer than those in serum. The cut-off values of NSE in serum and EBC determined in the present study were 14.30 and 4.65 ng/ml, respectively. The overall correct prediction percentage was 57.76% for serum-NSE and 59.01% for EBC-NSE, and NSE had the highest predictive positive rate in SCLC (62.50% in serum; 87.50% in EBC) [90]. Even though numerous studies have confirmed the diagnostic value of NSE in SCLC, it is not an ideal biomarker; its sensitivity is relatively low. Some authors regard that the combined diagnosis of marker combinations can effectively improve the accuracy of diagnosis. In SCLC patients, diagnostic utility of other tumour markers, such as haptoglobin, CEA and CYFRA 21-1 was also confirmed [91]. Wang et al. [92] proposed that the combined detection of Hp, CEA, NSE and CYFRA21-1 could significantly improve the sensitivity and specificity of lung cancer diagnosis and could be used for pathological typing. The combination of four tumour markers could produce a positive detection rate of 85.0%, which is significantly higher than that of any single test. An area under the ROC curve (AUC) analysis showed that the positive detection rate of Hp and CYFRA21-1 was higher than other markers for squamous cell carcinoma. In adenocarcinoma cases, the positive detection rate of CEA is higher than other markers. For small cell carcinoma, NSE has the highest positive detection rate [92]. Jiang et al. [93] also found that the detection of TK1 combined with CYFRA21-1, CEA and NSE increased the diagnostic value of TK1 for lung squamous cell carcinoma, adenocarcinoma and SCLC, respectively.  

**Monitoring treatment**

Increasing researches are focussed on the usefulness of various tumour markers in evaluating SCLC chemotherapy response. Liu et al. [94] investigated the associations of serum levels of lactate dehydrogenase (LDH), ProGRP and NSE with clinical response and survival in SCLC patients receiving first-line platinum-based chemotherapy. Of the 136 SCLC patients who received first-line platinum chemotherapy, 97 patients achieved complete relief (CR) and partial relief (PR), with an overall response rate of 71.3%. Compared with patients in stable disease (SD) and progress disease (PD), NSE and LDH level declined in patients who achieved CR + PR. Multivariate regression analysis revealed that NSE > 50.324 ng/ml and distant metastases were independent risk factors for patients achieving CR + PR and were independently correlated with worse overall survival (OS). The authors concluded that there is a promising role for NSE and LDH in predicting therapy response and survival of SCLC patients receiving first-line platinum-based chemotherapy. A lot of studies have observed that NSE levels are rapidly normalized in SCLC patients receiving combined chemo- and radiotherapy [85,95–97]. The reasons for the phenomenon are difficult to explain. Perhaps, it should be associated with the influence of chemotherapeutics on enolase activity and disorders of anaerobic glycolysis. Núria et al. have developed a model for biomarker that, without using tumour size data, is capable of predicting disease progression assessed by CT scans (RECIST data) in SCLC patients [98]. They successfully applied this framework to data regarding LDH and NSE concentrations in patients diagnosed with SCLC and believe that the proposed modelling framework of circulating biomarkers could constitute a powerful additional strategy for disease monitoring in SCLC patients.
Table 1 Basic information about the utility of NSE determinations in SCLC

| Utility of NSE                                                                 | References |
|-------------------------------------------------------------------------------|------------|
| Diagnosis                                                                    | [86,89,96,87] |
| In SCLC patients, serum NSE levels are significantly different due to tumour size, disease stage and distant metastasis | [88]       |
| Predictive value                                                             |            |
| NSE is a sensitive marker for therapeutic monitoring of lung cancer           | [96]       |
| Serial NSE measurements are advantageous for early prediction of relapse in SCLC patients | [101]     |
| At relapse, the serum level of NSE is a useful predictive marker for CR to salvage chemotherapy | [102]     |
| Prognostic value                                                             |            |
| In SCLC patients, NSE is a better prognostic factor in comparison with ProGRP | [85]       |
| For SCLC patients who did not receive treatment - the serum NSE level > 19.0 ng/ml shorter MS | [103]     |
| For SCLC patients receiving first-line platinum-based chemotherapy, NSE is of great predictive value for the therapy response | [94]       |
| Before treatment - high NSE serial concentration (>50.324 ng/ml) – unfavourable prognostic factor | [102]     |
| For patients with SCLC who have achieved a CR or PR to first-line chemotherapy, the serum level of NSE is a useful prognostic factor after relapse | [102]     |

Prognosis

Even though SCLC is initially sensitive to radiotherapy and chemotherapy, secondary multi-drug resistance is extremely easy to occur. The majority of patients have poor prognosis and eventually die of tumour recurrence. Data from the Moffitt Cancer Center showed that median survival (MS) time of patients with SCLC is poor, approximately 25.1 months in LD and 10.4 months in ED patients. Five-year survival rates are 9.9% and lower than 3% in LD and ED patients, respectively [99]. Petrovic et al. [84] analyzed the effects of circulating neuroendocrine markers CGA, ProGRP and NSE, in addition to other more classical prognostic variables, on survival duration of SCLC patients. MS of the entire study population (97 untreated patients from a single-centre and histologically proven SCLC, of which 51.5% were ED patients) was 13 months. Univariate Cox regression analysis found that survival rate significantly correlated with performance status (PS), disease stage, CGA, ProGRP and NSE levels, while age and sex did not affect prognosis. An ECOG performance status ≥ 2, extensive stage disease, a serum CGA level > 56 ng/ml, a serum ProGRP level > 58 pg/ml, and a serum NSE level > 19 ng/ml would lead to a shorter survival time of patients. The study carried out in 2016 by Huang et al. [85] supported the previous theory and emphasized NSE in serum as a better prognostic factor than Pro-GRP, because it is a prognostic factor independent of disease stage. According to some authors, an increase recurring in NSE level in serum of SCLC patients after chemotherapy may be related to the relapse of the disease. At relapse, 20–60% of patients had elevated serum levels of NSE [100–102], and the rate of CR to salvage chemotherapy in those patients was significantly lower than patients without elevated levels of NSE (2.2 vs 26.7%; P=0.001) [102]. These results show that serial NSE measurements are useful for the early prediction of SCLC relapse and for early administration of salvage chemotherapy for affected patients.

A series of studies document the value of NSE in the diagnosis, monitoring and evaluation of treatment response in SCLC patients, as well as the prognostic and predictive values of this biomarker. A summary of the information obtained both from literature and from our studies on the utility of NSE in diagnosing SCLC patients is contained in Table 1.

NSCLC

Although NSCLC is a common clinical pathological type of lung cancer, accounting for approximately 80–85%, its malignancy is relatively low. For early NSCLC cases, the 5-year survival rate can be significantly improved by standard surgical treatment [104]. However, it is difficult to make early diagnosis for NSCLC. Most patients are at advanced disease at diagnosis, thus missing the best time for radical surgery. Therefore, it is very important to establish a complete screening system for NSCLC for its early diagnosis and treatment [82,105,106]. As mentioned earlier, NSE is the first choice in tumour markers for the diagnosis of SCLC, but there are still partial NSCLC patients with elevated serum NSE levels clinically [107]. Dong et al. [108] found that CEA and NSE in serum are potentially effective biomarkers for diagnosing NSCLC. The diagnostic sensitivity and specificity were 66.67 and 78.69%, respectively, for serum NSE at the cut-off value of 19.35 ng/ml; the diagnostic area under the ROC curve was 0.76 for NSE. However, some studies have suggested that in patients with peripheral pulmonary carcinoma, the detection of tumour markers in BALF had more diagnostic value than serum samples [109]. In addition, some studies found that in patients with advanced NSCLC who have lost the opportunity for surgery and were treated with gefitinib (an epidermal growth
factor tyrosine kinase inhibitor, EGFR-TKI), the progression-free survival (PFS) and OS significantly reduced with increased in plasma NSE level before treatment, indicating that high pre-treatment serum level of NSE predicted poor EGFR-TKI therapeutic effect. Thus, it could be clinically useful in patients with NSCLC scheduled to receive gefitinib treatment [110,111]. At present, the value of NSE in judging prognosis of NSCLC is still controversial. Some believe that pre-treatment serum NSE level is an important prognostic factor of advanced NSCLC. In a recent study involving 224 patients, Wang et al. [112] evaluated the prognostic significance of serum tumour markers (CYFRA 21-1, CEA and NSE) in locally advanced squamous cell carcinoma of lung (LA-SCCL) after radiotherapy and found that increased NSE predicted poor distant metastasis-free survival (DMFS). Another analysis by Chen et al. [113] and Nisman et al.’s [103] team confirmed this conclusion, and found that a higher level of NSE before treatment was closely related to brain metastasis of advanced NSCLC. The serum level of NSE in 28 patients with brain metastasis was significantly higher than that of 98 patients without metastasis (34.18 ± 28.48 vs. 13.87 ± 4.49 ng/ml, P<0.05). Zhou et al. [114] also found that age, pathological type and serum NSE concentration of lung cancer patients are independent risk factors for bone metastasis. The NSE serum concentration of patients with bone lesions is significantly higher than those without bone metastasis (39.18 ± 62.18 vs. 29.16 ± 40.21 ng/ml, P<0.018) [114]. However, other studies have proposed that serum NSE level in NSCLC patients has no prognostic significance [115,116].

NSE and tuberculosis
As early as 2009, Racil et al. [117] proposed the diagnostic value of NSE in pulmonary tuberculosis (TB). They conducted a prospective study and collected serum levels of four tumour markers including NSE, cancer antigen 125 (CA125), ACE and CYFRA21-1 before anti-TB chemotherapy in 40 male TB patients (during 2005–2007). They found the levels of NSE were high in 91.66% of cases with an average value of 29.22 ng/ml (2.24 × normal). This highest sensitivity was superior to those of other tumour markers: 55.55% for CA125, 28.94% for ACE and 7.6% for CYFRA21-1. They thought the highest sensitivity of the NSE in pulmonary TB, with no neoplastic pathology could be interesting for diagnosis of smear negative TB, with small amounts of Bacilli. In addition, several studies have found that the levels of NSE, S100B and Neuropeptide Y (NPY) in serum and cerebrospinal fluid of children with acute miliary TB secondary to tuberculous meningitis are significantly higher than those of children with acute miliary TB or meningitis alone [118,119]. The early detection of these tumour markers is of great significance for the diagnosis of tuberculosis meningitis secondary to acute miliary TB.

Nam et al. [120] discussed the predictive value of NSE in indicating TB activity and severity and determined the origin of NSE in TB patients. They conducted a single-centre retrospective analysis of newly diagnosed TB patients from the years 2010 to 2011. According to chest X-ray, patients were divided into two disease groups (focal segmental or extensive). Pre- and post-treatment NSE concentrations were evaluated. In order to determine the origin of serum NSE, NSE staining was compared with macrophage-specific CD68 staining in lung tissue and tissue microarray using immunohistochemistry and immunofluorescence. The results showed that the serum concentration of NSE increased significantly in TB patients, and NSE level decreased after treatment (P<0.001). The average serum concentration of NSE in the extensive group (25.12 ng/ml) was significantly higher than that in the focal segment group (20.23 ng/ml, P=0.04). Immunohistochemical staining showed that a large number of macrophages were positive for NSE and CD68 in TB tissues. In addition, NSE signals mainly co-located with CD68 signals in tissue microarrays of TB patients. The authors regarded that NSE could be used to monitor TB activity and therapeutic response and that elevated serum NSE level is, at least in part, derived from macrophages in granulomatous lesions.

NSE and chronic obstructive pulmonary disease
Barouchos et al. [121] investigated the correlation between tumour markers and inflammatory biomarkers in patients with chronic obstructive pulmonary disease (COPD) exacerbation. Referring to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), patients were categorized into one of two disease groups (severity C and D). As a result, in group C, there was a significant positive correlation between C-reactive protein (CRP) and CA125 (P=0.05) while in group D, there was a significant positive correlation between white blood cells count (WBC) and NSE (P=0.02), between CRP and Cancer antigen 19-9 (CA19-9) (P=0.02) and NSE (P<0.001), and between the erythrocyte sedimentation rate (ESR) and NSE (P=0.03). In contrast, there was no significant difference in the two groups for NSE, CEA and CYFRA21-1. These results suggested that certain tumour markers were increased and were associated with increased levels of inflammatory biomarkers and with disease severity.
NSE and solitary pulmonary nodules
With the development of imaging technology and low-dose spiral CT chest screening technology, the number of solitary pulmonary nodules (SPNs) found in clinics is gradually increasing. The benign lesions of SPN are mostly infectious granuloma, followed by TB and hamartoma. The malignant lesions are mainly lung adenocarcinoma and squamous cell carcinoma. It is of great significance to identify the benign and malignant SPN as early as possible. Chu et al. [128] investigated the potential diagnostic values of CEA, SCC, CYFRA21-1 and NSE for lung cancer in patients with suspicious pulmonary masses in an extensive and large-scaled population. A combination assay of SCC, CEA, CYFRA21-1 and NSE increased sensitivity to 43.4%, which was still low in diagnosing lung cancer but was higher than any other single tumour marker. However, these results are inconsistent with that of Ni and Liu [123]. In their study, the difference in NSE positive rate and serum level between benign and malignant SPN had no statistical significance. The area under ROC curve was less than 0.5, which is not of diagnostic value for SPN. This difference could be due to the small proportion of SCLC in their cases.

NSE and pulmonary alveolar proteinosis
Pulmonary alveolar proteinosis (PAP) is an extremely rare interstitial lung disease characterized by the abnormal alveolar accumulation of a large number of lipoproteinaceous substances leading to impairment of lung ventilation function and increased risk of respiratory infections. The aetiology is still not clear and may be related to functional defects in alveolar macrophages. The diagnosis of PAP is easily missed or confused with other interstitial lung diseases with similar manifestations since it lacks typical clinical symptoms. Currently, open-lung biopsy and bronchopulmonary biopsy are used as the gold standard for clinical diagnosis but it is less required because BALF and imaging examination also have great significance for diagnosis. In 2016, Mo et al. [124] analyzed the clinical, pathological and biochemical characteristics of 11 patients with PAP in order to provide more information on diagnosis and management of PAP. They observed that CEA increased in most patients and CYFRA21-1 and NSE increased in all patients. Similar results were also obtained in a study by Fang et al. [125], in serum of patients with PAP, the changes of tumour markers (CEA, SCC and NSE) were consistent with the changes of severity index (LDH and PaO₂). Especially, significant positive correlations were found between levels of CEA and NSE in serum and LDH values ($r = 0.60$, $P<0.001$ and $r = 0.56$, $P<0.001$, respectively). After whole lung lavage (WLL), the levels of CEA, NSE and SCC in serum decreased significantly ($15.7 \pm 22$ vs. $8.7 \pm 10.6, 16.6 \pm 11.8$ vs. $7.9 \pm 5.2, 0.59 \pm 0.42$ vs. $0.4 \pm 0.24; P<0.05$ respectively). In addition, Arai et al. [126] also specifically analyzed the correlation between CYFRA21-1 and other disease severity markers of PAP, including pulmonary function parameters, alveolar–arterial oxygen gradient, British Medical Research Council score reflecting shortness of breath and disease severity score. They observed that serum CYFRA 21-1 level at diagnosis was significantly correlated with the measured disease severity parameters. Following WLL and granulocyte-macrophage colony-stimulating factor (GM-CSF) inhalation, serum CYFRA21-1 level was significantly reduced, and immunohistochemistry showed CYFRA 21-1 was localized in hyperplastic alveolar type II cells and lipoprotein substances in alveoli. Thus, the serum levels of tumour markers such as CEA, NSE, SCC and CYFRA 21-1 may reflect the severity of disease and predict the therapeutic effect of WLL.

NSE and acute lung injury
Recently, Gong et al. [127] found that both the expression and activity of PFKFB3, a key glycolytic activator, were markedly increased in lung endothelial cells (ECs) of mice challenged with lipopolysaccharide (LPS) in vitro and in LPS-treated human pulmonary arterial ECs (HPAECs) in vitro. And blockage of glycolysis by targeting PFKFB3 alleviates sepsis-related acute lung injury (ALI) via suppressing inflammation and apoptosis of ECs. Consistently, the research of Zhong et al. [128] also demonstrated inhibition of glycolysis alleviates LPS-induced ALI in a mouse model in 2016. Similarly, we believe NSE, as an indispensable enzyme in glycolytic pathway, may play a key pathogenic role in ALI. Our research group verified this idea in SD rat model of ALI induced by severe acute pancreatitis (SAP-ALI). Severe acute pancreatitis (SAP) is an acute abdominal disease with many complications and high mortality [129]. SAP is often complicated with systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), with mortality as high as 10–30% [130]. ALI is one of the most common early complications of SAP and is also the main reason for high mortality in the early stage. Sixty percent of SAP patients die of respiratory failure within 7 days after admission in hospital [131,132]. In recent years, scholars have defined this lung damage as SAP-ALI. Although many researches have focussed on the pathogenesis and drug intervention of SAP-ALI, the exact pathogenesis is still not fully clear, and the mortality rate is still high. We found that in SAP-ALI rat model, the expressions of NSE, caspase-1, IL-1β and TNF-α in injured lung tissue were significantly increased, and NSE inhibitor could significantly inhibit the expression and activation of caspase-1 and alleviate lung injury. This may be related to
the abnormal enhancement of glycolysis mediated by NSE (a key isoenzyme of glycolysis) and the promotion of cell pyroptosis mediated by caspase-1 during SAP-ALI [133].

**Conclusion**

NSE is considered a multifunctional protein and different cellular localization and interactions with other molecules strongly suggest its multiple cellular engagements: prompting that its traditional name as ‘neuron specific’ may need to be revisited. Increasing empirical evidence show that NSE can play important role in the diagnosis, treatment monitoring and prognosis evaluation of various lung diseases. Advanced understanding of the structure, function, biochemical and clinical characteristics of NSE may provide new ideas for the treatment of these diseases.

**Funding**

This study was supported by the Natural National Science Foundation of China [grant number 81573751] and Natural Science Foundation Guidance Plan of Liaoning Province [grant number 2019-ZD-0919].

**Competing Interests**

The authors declare that there are no competing interests associated with the manuscript.

**Abbreviations**

ACE, antigen carcino embryonnaire; ALI, acute lung injury; ALP, alkaline phosphatase; BALF, bronchoalveolar lavage fluid; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; CGA, chromogranin A; CI, confidence interval; CR, complete relief; CRP, C-reactive protein; CV, coefficient of variation; EBC, exhaled breath condensate; ECM, extracellular matrix; ECOG, Eastern Cooperative Oncology Group; ED, extensive-stage disease; EGFR-TKI, epidermal growth factor tyrosine kinase inhibitor; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; Eno1, α-enolase; FITC, fluorescein isothiocyanate; INF-γ, interferon-gamma; LD, limited-stage disease; LDH, lactate dehydrogenase; LOD, limit of detection; LPS, lipopolysaccharide; mAb, monoclonal antibody; MBP-1, c-myc promoter-binding protein-1; MCP-1, monocyte chemoattractant protein-1; MS, median survival; NNE, non-neuronal enolase; NO, nitrogenmonoxide; NSCLC, non-small cell lung cancer; NSE, neuron-specific enolase; OS, overall survival; PAP, pulmonary alveolar proteinosis; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PR, partial relief; ProGRP, pro-gastrin-releasing peptide; RECIST, Response Evaluation Criteria in Solid Tumors; RIA, radioimmunoassay; ROC, receiver operating characteristic; ROS, reactive oxygen species; SAP-ALI, ALI induced by severe acute pancreatitis; SCLC, small cell lung cancer; SD, stable disease; SPN, solitary pulmonary nodule; TB, tuberculosis; TNM, tumor node metastasis; WLL, whole lung lavage.

**References**

1. Piast, M., Kustrzeba-Wojcicka, I., Matusiewicz, M. and Banas, T. (2005) Molecular evolution of enolase. *Acta Biochim. Pol.* **52**, 507–513
2. Hannaaert, V., Brinkmann, H., Nowitzki, U. et al. (2000) Enolase from *Trypanosoma brucei*, from the amitochondriate protist *Mastigamoeba balamuthi*, and from the chloroplast and cytosol of *Euglena gracilis*: pieces in the evolutionary puzzle of the euarkytic glycolytic pathway. *Mol. Biol. Evol.* **17**, 989–1000, [https://doi.org/10.1093/oxfordjournals.molbev.a026395](https://doi.org/10.1093/oxfordjournals.molbev.a026395)
3. Seki, S.M. and Gaultier, A. (2017) Exploring non-metabolic functions of glycolytic enzymes in immunity. *Front. Immunol.* **8**, 1549, [https://doi.org/10.3389/fimmu.2017.01549](https://doi.org/10.3389/fimmu.2017.01549)
4. Capello, M., Ferri-Borgogno, S., Cappello, P. and Novelli, F. (2011) α-enolase: a promising therapeutic and diagnostic tumor target. *FEBS J.* **278**, 1064–1074, [https://doi.org/10.1111/j.1742-4658.2011.08025.x](https://doi.org/10.1111/j.1742-4658.2011.08025.x)
5. Rider, C.C. and Taylor, C.B. (1975) Enolase isoenzymes. II. hybridization studies, developmental and phylogenetic aspects. *Biochim. Biophys. Acta* **405**, 175–187, [https://doi.org/10.1016/0005-2795(75)90328-1](https://doi.org/10.1016/0005-2795(75)90328-1)
6. Brewer, J.M. and Ellis, P.D. (1983) 31P-nmr studies of the effect of various metals on substrate binding to yeast enolase. *J. Inorg. Biochem.* **18**, 71–82, [https://doi.org/10.1016/0162-0134(83)80041-7](https://doi.org/10.1016/0162-0134(83)80041-7)
7. Fletcher, L., Rider, C.C. and Taylor, C.B. (1976) Enolase isoenzymes. III. Chromatographic and immunological characteristics of rat brain enolase. *Biochim. Biophys. Acta* **452**, 245–252, [https://doi.org/10.1016/0005-2744(76)90077-2](https://doi.org/10.1016/0005-2744(76)90077-2)
8. Giallongo, A., Feo, S., Moore, R., Croce, C.M. and Showe, L.C. (1986) Molecular cloning and nucleotide sequence of a full-length cDNA for human α-enolase. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6741–6745, [https://doi.org/10.1073/pnas.83.18.6741](https://doi.org/10.1073/pnas.83.18.6741)
9. Feo, S., Oliva, D., Barbieri, G., Xu, W.M., Fried, M. and Giallongo, A. (1990) The gene for the muscle-specific enolase is on the short arm of human chromosome 17. *Genomics* **6**, 192–194, [https://doi.org/10.1016/0888-7543(90)90467-9](https://doi.org/10.1016/0888-7543(90)90467-9)
10. Pancholi, V. (2001) Multifunctional α-enolase: its role in diseases. *Cell. Mol. Life Sci.* **58**, 902–920, [https://doi.org/10.1007/PL00000910](https://doi.org/10.1007/PL00000910)
11. Pancholi, V. and Fischetti, V.A. (1998) α-enolase, a novel strong plasminogenbinding protein on the surface of pathogenic streptococci. *J. Biol. Chem.* **273**, 14503–14515, [https://doi.org/10.1074/jbc.273.23.14503](https://doi.org/10.1074/jbc.273.23.14503)
12. Marangos, P.J., Schmechel, D., Zis, A.P. and Goodwin, F.K. (1979) The existence and neurobiological significance of neuronal and glial forms of the glycolytic enzyme enolase. *Biol. Psychiatry* **14**, 563–579

© 2019 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
13 Marangos, P.J., Schmechel, D.E., Parma, A.M. and Goodwin, F.K. (1980) Developmental profile of neuron-specific (NSE) and non-neuronal (NNE) enolase. *Brain Res.* **190**, 185–193, https://doi.org/10.1016/0006-8993(80)91168-3

14 Schmechel, D.E., Brightman, M.W. and Marangos, P.J. (1980) Neurons switch from non-neuronal enolase to neuron-specific enolase during differentiation. *Brain Res.* **190**, 195–214, https://doi.org/10.1016/0006-8993(80)91169-5

15 Marangos, P.J., Zis, A.P., Clark, R.L. and Goodwin, F.K. (1978) Neuronal, non-neuronal and hybrid forms of enolase in brain: structural, immunological and functional comparisons. *Brain Res.* **150**, 117–133, https://doi.org/10.1016/0006-8993(78)90657-1

16 Schmechel, D., Marangos, P.J., Zis, A.P., Brightman, M. and Goodwin, F.K. (1978) Brain endolases as specific markers of neuronal and glial cells. *Science* **199**, 313–315, https://doi.org/10.1126/science.339349

17 Marangos, P.J. and Schmechel, D.E. (1987) Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine tissues. *Ann. Rev. Neurosci.* **10**, 269–295, https://doi.org/10.1146/annurev.ne.10.030187.001413

18 Nakatsuka, S., Nishiu, M., Tomita, Y. et al. (2002) Enhanced expression of neuron-specific enolase (NSE) in pyothorax-associated lymphoma (PAL). *Jpn. J. Cancer Res.* **93**, 411–416, https://doi.org/10.1111/j.1349-7006.2002.tb01272.x

19 Leiherer, A., Stoemer, K., Muendlein, A. et al. (2016) Quercetin impacts expression of metabolism- and obesity-associated genes in SGBS adipocytes. *Nutrients* **8**, 282, https://doi.org/10.3390/nu8050282

20 Haimoto, H., Takahashi, Y., Koshikawa, T., Nagura, H. and Kato, K. (1985) Immunohistochemical localization of gamma-enolase in normal human tissues other than nervous and neuroendocrine tissues. *Lab. Invest.* **52**, 257–263

21 Nakajima, K., Hamanoue, M., Takemoto, N., Hattori, T., Kato, K. and Kohsaka, S. (1994) Plasminogen binds specifically to alpha-enolase on rat neuronal plasma membrane. *J. Neurochem.* **63**, 2048–2057, https://doi.org/10.1002/jnc.4706306048

22 Redlitz, A., Fowler, B.J., Plow, E.F. and Miles, L.A. (1995) The role of an enolase-related molecule in plasminogen binding to cells. *Eur. J. Biochem.* **227**, 407–415, https://doi.org/10.1111/j.1422-1321.1995.tb13111.x

23 Dudani, A.K., Cummings, C., Hashemi, S. and Ganz, P.R. (1993) Isoform of enolase specific for platelets and human endothelial cells. *Thromb. Res.* **69**, 185–196, https://doi.org/10.1016/0049-3848(93)90044-0

24 Hisa, K.C., Shih, N.Y., Fang, H.L. et al. (2013) Surface alpha-enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. *PLoS ONE* **8**, e69354, https://doi.org/10.1371/journal.pone.0069354

25 Lopez-Alemany, R., Suelves, M. and Munoz-Canoves, P. (2003) Plasmin generation dependent on alpha-enolase-type plasminogen receptor is required for myogenesis. *Thromb. Haemost.* **90**, 724–733, https://doi.org/10.1160/TH03-04-0291

26 Lopez-Alemany, R., Longstaff, C., Hawley, S. et al. (2003) Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-enolase. *Am. J. Hematol.* **72**, 234–242, https://doi.org/10.1002/ajh.10299

27 Haque, A., Ray, S.K., Cox, A. and Banik, N.L. (2016) Neuron specific enolase: a promising therapeutic target in acute spinal cord injury. *Metab. Brain Dis.* **31**, 487–495, https://doi.org/10.1007/s11011-016-9801-6

28 Feo, S., Arcuri, D., Pillidini, E., Passantino, R. and Giallongo, A. (2000) EN01 gene product binds to the c-myc promoter and acts as a transcriptional repressor: relationship with Myc promoter-binding protein 1 (MBP-1). *FEBS Lett.* **473**, 47–52, https://doi.org/10.1016/S0014-5793(00)01494-0

29 Subramanian, A. and Miller, D.M. (2000) Structural analysis of alpha-enolase. Mapping the functional domains involved in down-regulation of the c-myc protooncogene. *J. Biol. Chem.* **275**, 5958–5965, https://doi.org/10.1074/jbc.275.8.5958

30 Iida, H. and Yahara, I.J.N. (1985) Yeast heat-shock protein of M r 48,000 is an isoprotein of enolase. *Nature* **315**, 688–690

31 Aaronson, R.M., Graven, K.K., Tucci, M., McDonald, R.J. and Farber, H.W. (1995) Non-neuronal enolase is an endothelial hypoxic stress protein. *Am. J. Hematol.* **52**, 527–535, https://doi.org/10.1002/ajh.10299

32 Haque, A., Polycn, R., Mazzelle, D. and Banik, N.L. (2018) New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. *Brain Sci.* **8**, 33, https://doi.org/10.3390/brainsci8020033

33 Moore, B.W. and McGregor, D. (1965) Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. *J. Biol. Chem.* **240**, 1647–1653

34 Marangos, P.J., Parma, A.M. and Goodwin, F.K. (1978) Functional properties of neuronal and glial isoenzymes of brain enolase. *J. Neurochem.* **31**, 727–733, https://doi.org/10.1111/j.1471-4159.1978.tb07847.x

35 Soh, M.A., Garrett, S.H., Somji, S. et al. (2011) Arsenic, cadmium and neuron specific enolase (EN02, gamma-enolase) expression in breast cancer. *Cancer Lett.* **11**, 41, https://doi.org/10.1186/1475-2867-11-41

36 Cao, F., Yang, X.F., Liu, W.G. et al. (2008) Expression of neuron-specific enolase and S-100beta protein level in experimental acute spinal cord injury. *J. Clin. Neurosci.* **15**, 541–544, https://doi.org/10.1016/j.jocn.2007.05.014

37 Haque, A., Polycn, R., Mazzelle, D. and Banik, N.L. (2017) Neuron-specific enolase (NSE) in neurodegeneration and neuroprotection. *Brain Sci.* **8**, 33, https://doi.org/10.3390/brainsci8020033

38 Iida, H. and Yahara, I.J.N. (1985) Yeast heat-shock protein of Mr 48,000 is an isoprotein of enolase. *Nature* **315**, 688–690

39 Schmechel, D.E., Brightman, M.W. and Marangos, P.J. (1980) Neurons switch from non-neuronal enolase to neuron-specific enolase during differentiation. *Brain Res.* **190**, 195–214, https://doi.org/10.1016/0006-8993(80)91169-5

40 Zelen, M. (1973) Keynote address on biostatistics and data retrieval. *Cancer Chemother. Rep.* **4**, 31–42

41 Hattori, T., Ohsawa, K., Mizuho, Y., Kato, K. and Kohsaka, S. (1994) Synthetic peptide corresponding to 30 amino acids of the C-terminal of neuron-specific enolase and S-100beta protein level in experimental acute spinal cord injury. *J. Neurochem.* **63**, 2048–2057, https://doi.org/10.1002/jnc.4706306048

42 Obermajer, N., Doljak, B., Jamnik, P., Fonovic, U.P. and Kos, J. (2009) Cathepsin X cleaves the C-terminal dipeptide of alpha- and gamma-enolase and impairs survival and neuritogenesis of neuronal cells. *Int. J. Biochem. Cell Biol.* **41**, 1685–1696, https://doi.org/10.1016/j.biocel.2009.02.019
43 Hafner, A., Obermajer, N. and Kos, J. (2010) Gamma-1-syntrophin mediates trafficking of gamma-enolase towards the plasma membrane and enhances its neurotrophic activity. Neurosignals 18, 246–258, https://doi.org/10.1007/s00034-009-0128-0

44 Hafner, A., Glavan, G., Obermajer, N., Zivin, M., Schliebs, R. and Kos, J. (2013) Neuronal protective role of neuron-specific enolase in microglia in a mouse model of Alzheimer’s disease is regulated by calcineurin. Aging Cell 12, 604–614, https://doi.org/10.1111/acel.12093

45 Pislar, A.H. and Kos, J. (2013) C-terminal peptide of gamma-enolase impairs amyloid-beta-induced apoptosis through p75NTR signaling. Neuronol. Med. 15, 623–635, https://doi.org/10.1016/j.surnm.2013.06.007

46 Miles, L.A., Dahlgren, C.M., Plescia, J., Felez, J., Kato, K. and Plow, E.F. (1991) Role of cell-surface lysines in plasminogen binding to cells: identification of alpha-enolase as a candidate plasmingen receptor. Biochemistry 30, 1682–1691, https://doi.org/10.1021/bi00220a034

47 Butterfield, D.A. and Lange, M.L. (2009) Multifunctional roles of enolase in Alzheimer’s disease brain: beyond altered glucose metabolism. J. Neurochem. 111, 915–933, https://doi.org/10.1111/j.1471-4159.2009.06397.x

48 Soh, M., Dunlevy, J.R., Garrett, S.H. et al. (2012) Increased neuron specific enolase expression by urethelial cells exposed to or malignantly transformed by exposure to Cd(2+) or As(3+). Toxicol. Lett. 212, 66–74, https://doi.org/10.1016/j.toxlet.2012.05.003

49 Sanders, E. and Diehl, S. (2015) Analysis and interpretation of transcriptomic data obtained from extended Warburg effect genotypes in patients with clear cell renal cell carcinoma. Oncoscience 2, 151–166, https://doi.org/10.18632/oncoscience.128

50 Annila, S. and Widmann, C. (2010) Glucose metabolism in cancer cells. Curr. Opin. Clin. Nutr. Metab. Care 13, 466–470, https://doi.org/10.1097/MCO.0b013e3283a5577

51 Bose, S. and Le, A. (2016) Glucose metabolism in cancer. Adv. Exp. Med. Biol. 1063, 3–12, https://doi.org/10.1007/978-3-319-77736-8_1

52 Zivin, T. and Kos, J. (2015) Gamma-enolase: a well-known tumour marker, with a less-known role in cancer. Radiol. Oncol. 49, 217–226, https://doi.org/10.1515/raon-2015-0035

53 Yan, T., Skaffnesmo, K.O., Leiss, L. et al. (2011) Neuronal markers are expressed in human gliomas and NSE knockdown sensitizes glioblastoma cells to radiotherapy and temozolomide. BMC Cancer 11, 524, https://doi.org/10.1186/1471-2407-11-524

54 Levin, V.A., Panchabhai, S.C., Shen, L., Kornblau, S.M., Qiu, Y. and Baggerly, K.A. (2010) Different changes in protein and phosphoprotein levels result from serum starvation of high-grade glioma and adenocarcinoma cell lines. J. Proteome Res. 9, 179–191, https://doi.org/10.1021/pr900392b

55 Levin, V.A., Panchabhai, S., Shen, L. and Baggerly, K.A. (2012) Protein and phosphoprotein levels in glioma and adenocarcinoma cell lines grown in normoxia and hypoxia in monolayer and three-dimensional cultures. Proteome Sci. 10, 5, https://doi.org/10.1186/1477-5956-10-5

56 Dogterom, M. and Koenderink, G.H. (2019) Actin-microtubule crosstalk in cell biology. Nat. Rev. Mol. Cell Biol. 20, 38–54, https://doi.org/10.1038/s41580-018-0067-8

57 Hafner, A., Obermajer, N. and Kos, J. (2012) Gamma-Enolase C-terminal peptide promotes cell survival and neurite outgrowth by activation of the PI3K/Akt and MAPK/ERK signalling pathways. Biochem. J. 443, 439–450, https://doi.org/10.1042/BJ20111351

58 Marangos, P.J., Schmechel, D., Parma, A.M., Clark, R.L. and Goodwin, F.K. (1979) Measurement of neuron-specific (NSE) and non-neuronal (NNE) isoenzymes of enolase in rat, monkey and human nervous tissue. J. Neurochem. 33, 319–329, https://doi.org/10.1111/j.1471-4159.1979.tb1735.x

59 Marangos, P.J., Zomzely-Neurath, C. and York, C. (1975) Immunological studies of a nerve specific protein. Arch. Biochem. Biophys. 170, 289–293, https://doi.org/10.1016/0003-9861(75)90119-8

60 Parma, A.M., Marangos, P.J. and Goodwin, F.K. (1981) A more sensitive radioimmunoassay for neuron-specific enolase suitable for cerebrospinal fluid determinations. J. Neurochem. 36, 1093–1096, https://doi.org/10.1111/j.1471-4159.1981.tb01704.x

61 Paus, E. and Nustad, K. (1989) Immunoradiometric assay for alpha-gamma- and gamma-gamma-enolase (neuron-specific enolase), with use of monoclonal antibodies and magnetizable polymer particles. Clin. Chem. 35, 2034–2038

62 Body, J.J., Paesmans, M., Sculier, J.P. et al. (1992) Monoclonal immunoradiometric assay and polyclonal radioimmunoassay compared for measuring neuron-specific enolase in patients with lung cancer. Clin. Chem. 38, 748–751

63 Anastasiades, K.D., Mullins, R.E. and Conn, R.B. (1987) Neuron-specific enolase. Assessment by ELISA in patients with small cell lung cancer. J. Am. Clin. Pathol. 87, 245–249, https://doi.org/10.1093/jac/87.2.245

64 Kimura, S., Uchikawa, H., Yamamoto, R. and Kato, K. (1984) Practicable enzyme immunoassay for neuron-specific enolase in human serum. J. Appl. Biochem. 6, 319–324

65 Schmitt, U.M., Stieber, P., Hasholzner, U., Pahl, H., Hofmann, K. and Fateh-Moghadam, A. (1996) Methodological and clinical evaluation of two automated enzymatic immunoassays as compared with a radioimmunoassay for neuron-specific enolase. Eur. J. Clin. Chem. Clin. Biochem. 34, 679–682

66 Muley, T., Ebert, W., Stieber, P. et al. (2003) Technical performance and diagnostic utility of the new Elecsys neuron-specific enolase enzyme immunoassay. Clin. Chem. Lab. Med. 41, 95–103, https://doi.org/10.1515/CCLM.2003.017

67 Wevers, R.A., Jacobs, A.A. and Hommes, O.R. (1983) A bioluminescent assay for enolase (EC 4.2.1.11) activity in human serum and cerebrospinal fluid. Clin. Chim. Acta 135, 159–168, https://doi.org/10.1016/0003-9881(83)90131-6

68 Gerbitz, K.D., Sumner, J. and Thalheimer, J. (1984) Brain-specific proteins: solid-phase immunobioluminescence assay for neuron-specific enolase in human plasma. Clin. Chem. 30, 382–386

69 Viallard, J.L., Murthy, M.R., Betal, G. and Dastuque, B. (1986) Determination of serum neuron-specific enolase by differential immunocomplexation. Clin. Chim. Acta 161, 1–10, https://doi.org/10.1016/S0003-9881(86)80257-3

70 Viallard, J.L., Ven Murthy, M.R. and Dastuque, B. (1986) Rapid electrophoretic determination of neuron-specific enolase isoenzymes in serum. Clin. Chem. 32, 593–597

71 Fu, Y., Meng, M., Zhang, Y., Yin, Y., Zhang, X. and Xi, R. (2012) Chemiluminescence enzyme immunoassay using magnetic nanoparticles for detection of neuron specific enolase in human serum. Anal. Chem. 722, 114–118, https://doi.org/10.1021/ac2002007

72 Ho, J.A., Chang, H.C., Shih, N.Y. et al. (2010) Diagnostic detection of human lung cancer-associated antigen using a gold nanoparticle-based electrochemical immunoassay. Anal. Chem. 82, 5944–5950, https://doi.org/10.1021/ac1001959
76 Zhang, Q., Li, X., Qian, C., Dou, L., Cui, F. and Chen, X. (2018) Label-free electrochemical immunoassay for neuron-specific enolase based on 3D macroporous reduced graphene oxide/polyaniline film. Anal. Biochem. 540–541, 1–8

75 Wang, H., Han, H. and Ma, Z. (2017) Conductive hydrogel composed of 1,3,5-benzenetricarboxylic acid and Fe(3+) used as enhanced electrochemical immunosensing substrate for tumor biomarker. Bioelectrochemistry 114, 48–53, https://doi.org/10.1016/j.bioelechem.2016.12.006

74 Zhao, X., Qiao, L., Stauffer, G., Liu, B. and Girault, H.H. (2018) On-Chip spherophane nanoelectrospray ionization mass spectrometry for sensitive biomarker detection in small volumes. J. Am. Soc. Mass Spectrom. 29, 1538–1545, https://doi.org/10.1021/acs.jamss.8b00197

72 Liu, L., Wu, S., Jing, F. et al. (2016) Bead-based microarray immunoassay for lung cancer biomarkers using quantum dots as labels. Biosens. Bioelectron. 80, 300–306, https://doi.org/10.1016/j.bios.2016.01.084

71 Fitzmaurice, C., Akinyemiju, T.F., Global Burden of Disease Collaboration et al. (2018) Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol. 4, 1553–1568, https://doi.org/10.1001/jamaoncol.2018.2706

70 American Society of Clinical Pathologists (1982) The World Health Organization histological typing of lung tumours: second edition. Am. J. Clin. Pathol. 77, 123–136, https://doi.org/10.1093/ajcp/77.2.123

69 Siegel, R.L., Miller, K.D. and Jemal, A. (2018) Cancer statistics, 2018. CA Cancer J. Clin. 68, 7–30, https://doi.org/10.3322/caac.21442

68 Detterbeck, F.C., Postmus, P.E. and Tanoue, L.T. (2013) The stage classification of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 143, e191S–e210S, https://doi.org/10.1378/chest.12-2354

67 Petrovic, M., Bukumiric, Z., Zdravkovic, V., Mitrovic, S., Atkinson, H.D. and Jurisic, V. (2014) The prognostic significance of the circulating neurendocrine markers chromogranin A, pro-gastrin-releasing peptide, and neuron-specific enolase in patients with small-cell lung cancer. J. Thorac. Oncol. 9, 823, https://doi.org/10.1097/JTO.0000000000000313

66 Huang, Z., Xu, D., Zhang, F., Ying, Y. and Song, L. (2016) Pro-gastrin-releasing peptide and neuron-specific enolase: useful predictors of response to chemotherapy and survival in patients with small-cell lung cancer. Clin. Transl. Oncol. 18, 1019–1025, https://doi.org/10.1007/s12094-015-1479-4

65 Kostovski, M. and Petrusheska, G. (2014) Antigenic phenotype of lung carcinomas: usual spectrum of distribution of thyroid transcription factor-1, cytokeratin 7, cytokeratin 20, and neuron specific enolase–basic immunohistochemical study of 21 cases. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 35, 199–207

64 Fujita, K., Haimoto, H., Imaizumi, M., Abe, T. and Kato, K. (1987) Evaluation of γ-enolase as a tumor marker for lung cancer. Cancer 60, 362–369, https://doi.org/10.1002/1097-0142(19870801)60:3%3c362::AID-CNCR2820600313%3e3.0.CO;2-U

63 Cooper, E.H., Splinter, T.A., Brown, D.A., Muers, M.F., Peake, M.D. and Pearson, S.L. (1985) Evaluation of a radioimmunoassay for neuron specific enolase in small cell lung cancer. Br. J. Cancer 52, 333–338, https://doi.org/10.1038/bjc.1985.198

62 Huang, L., Zhou, J.G., Yao, W.X. et al. (2017) Systematic review and meta-analysis of the efficacy of serum neuron-specific enolase for early small cell lung cancer screening. Oncotarget 8, 64356–64372

61 Zou, Y., Wang, L., Zhao, C. et al. (2013) CEA, SCC and NSE levels in exhaled breath condensate–possible markers for early detection of lung cancer. Breath Res. 7, 047101, https://doi.org/10.1080/17527155.2014.947101

60 Shi, N., Deng, L., Chen, W. et al. (2017) Is microRNA-127 a novel biomarker for acute pancreatitis with lung injury? Dis. Markers 2017, 2017, 1–8, https://doi.org/10.1155/2017/124295

59 Wang, B., He, Y.J., Tian, Y.X., Yang, R.N., Zhu, Y.R. and Qiu, H. (2014) Clinical utility of haptoglobin in combination with CEA, NSE and CYFRA21-1 for diagnosis of lung cancer. Asian Pac. J. Cancer Prev. 15, 9611–9614, https://doi.org/10.7314/APJCP.2014.15.22.9611

58 Jiang, Z.F., Wang, M. and Xu, J.L. (2018) Thymidine kinase 1 combined with CYFRA21-1 and NSE improved its diagnostic value for lung cancer. Life Sci. 194, 1–6, https://doi.org/10.1016/j.lfs.2017.10.020

57 Liu, X., Zhang, W., Yin, W. et al. (2017) The prognostic value of the serum neuron-specific enolase and lactate dehydrogenase in small cell lung cancer patients receiving first-line platinum-based chemotherapy. Medicine (Baltimore) 96, e6285, https://doi.org/10.1097/MD.0000000000006285

56 Wojcik, E., Kulpa, J.K., Sas-Korzyczna, B., Korzeniowski, S. and Jakubowicz, J. (2008) ProGRP and NSE in therapy monitoring in patients with small cell lung cancer. Anticancer Res. 28, 3027–3033

55 Wang, L., Wang, D., Cheng, G. et al. (2016) Clinical evaluation and therapeutic monitoring value of serum tumor markers in lung cancer. Int. J. Biol. Markers 31, 860–867, https://doi.org/10.5301/jbm.5000177

54 Holdenrieder, S., von Pawel, J., Dankelmann, E. et al. (2008) Nucleosomes, ProGRP, NSE, CYFRA 21-1, and CEA in monitoring first-line chemotherapy of small cell lung cancer. Clin. Cancer Res. 14, 7813–7821, https://doi.org/10.1186/1078-0432.CCR-08-0678

53 Buil-Bruna, N., Lopez-Picazo, J.M., Moreno-Jimenez, M., Martin-Algarra, S., Ribba, B. and Troconiz, I.F. (2014) A population pharmacodynamic model for lactate dehydrogenase and neuron specific enolase to predict tumor progression in small cell lung cancer patients. AAPS J. 16, 609–619, https://doi.org/10.1208/s12248-014-9600-0

52 Schabath, B.S., Nguyen, A., Wilson, P., Sommerer, K.R., Thompson, Z.J. and Chiappori, A.A. (2014) Temporal trends from 1986 to 2008 in overall survival of small cell lung cancer patients. Lung Cancer 86, 14–21, https://doi.org/10.1016/j.lungcan.2014.07.014
100 Niho, S., Nishiwaki, Y., Goto, K. et al. (2000) Significance of serum pro-gastrin-releasing peptide as a predictor of relapse of small cell lung cancer: comparative evaluation with neuron-specific enolase and carcinoembryonic antigen. *Lung Cancer* **27**, 159–167, https://doi.org/10.1016/S0169-5002(99)00100-2

101 Nitta, T., Fukushima, M., Masuda, N. et al. (1995) Significance of serum neuron-specific enolase as a predictor of relapse of small cell lung cancer. *Jpn. J. Clin. Oncol.* **25**, 179–183

102 Hirose, T., Okuda, K., Yamaoka, T. et al. (2011) Are levels of pro-gastrin-releasing peptide or neuron-specific enolase at relapse prognostic factors after relapse in patients with small-cell lung cancer? *Lung Cancer* **71**, 224–228, https://doi.org/10.1016/j.lungcan.2010.05.004

103 Nisman, B., Heching, N., Biran, H., Barak, V. and Peretz, T. (2006) The prognostic significance of circulating neuroendocrine markers chromogranin a, pro-gastrin-releasing peptide and neuron-specific enolase in patients with advanced non-small cell lung cancer. *Tumour Biol.* **27**, 8–16, https://doi.org/10.1159/000090015

104 Schabath, M.B., Thompson, Z.J. and Gray, J.E. (2014) Temporal trends in demographics and overall survival of non-small-cell lung cancer patients at Moffitt Cancer Center from 1986 to 2008. *Cancer Control.* **21**, 51–56, https://doi.org/10.1177/10732748140211007

105 She, J., Yang, P., Hong, Q. and Bai, C. (2013) Lung cancer in China: challenges and interventions. *Chest* **143**, 1117–1126, https://doi.org/10.1378/chest.11-2948

106 Wu, W.S. and Chen, Y.M. (2014) Re-treatment with EGFR-TKIs in NSCLC patients who developed acquired resistance. *J. Pers. Med.* **4**, 297–310, https://doi.org/10.3390/jpm4030297

107 Ferrigno, D., Buccheri, G. and Giordano, C. (2003) Neuron-specific enolase is an effective tumour marker in non-small cell lung cancer (NSCLC). *Lung Cancer* **41**, 311–320, https://doi.org/10.1016/S0169-5002(03)00232-0

108 Dong, Y., Zheng, X., Yang, Z. et al. (2016) Serum carcinoembryonic antigen, neuron-specific enolase as biomarkers for diagnosis of non-small cell lung cancer. *J. Cancer Res. Ther.* **12**, 34–36, https://doi.org/10.1016/j.jjcr.2013.05.008

109 Zhang, S., Zhao, Y.F., Zhang, M.Z. and Wu, X.L. (2017) The diagnostic value of tumor markers in bronchoalveolar lavage fluid for the peripheral pulmonary carcinoma. *Clin. Respir. J.* **11**, 481–488, https://doi.org/10.1111/cij.12362

110 Inomata, M., Hayashi, R., Yamamoto, A. et al. (2015) Plasma neuron-specific enolase level as a prognostic marker in patients with non-small cell lung cancer receiving gefitinib. *Mol. Clin. Oncol.* **8**, 802–806, https://doi.org/10.3892/mco.2015.568

111 Fiala, O., Pesek, M., Fink, J. et al. (2014) The role of neuron-specific enolase (NSE) and thymidine kinase (TK) levels in prediction of efficacy ofEGFR-TKIs in patients with advanced-stage NSCLC [corrected]. *Anticancer Res.* **34**, 5193–5198

112 Wang, J., Jiang, W., Zhang, T. et al. (2018) Increased CYFRA 21-1, CEA and NSE are prognostic of poor outcome for locally advanced squamous cell carcinoma in lung: A Nomogram and Recursive Partitioning Risk Stratification Analysis. *Transl. Oncol.* **11**, 999–1006, https://doi.org/10.1016/j.tranon.2018.05.008

113 Chen, Y., Peng, W., Huang, Y. et al. (2015) Significance of serum neuron-specific enolase before treatment in predicting brain metastases and prognosis of advanced non-small cell lung cancer. *Zhong. Zhong Liu Za Zhi* **37**, 508–511

114 Zhou, Y., Chen, W.Z., Peng, A.F., Tong, W.L., Liu, J.M. and Liu, Z.L. (2017) Neuron-specific enolase, histopathological types, and age as risk factors for bone metastases in lung cancer. *Tumour Biol.* **39**, 10428317714194, https://doi.org/10.1007/s13277-017-4194

115 Tiseo, M., Ardizzoni, A., Cafferata, M.A. et al. (2006) Predictive and prognostic significance of neuron-specific enolase (NSE) in non-small cell lung cancer. *Anticancer Res.* **28**, 507–513

116 Yan, H.J., Tan, Y. and Gu, W. (2014) Neuron specific enolase and prognosis of non-small cell lung cancer: a systematic review and meta-analysis. *J. BUON* **19**, 153–156

117 Racil, H., Saad, S., Rouhou, S.C. et al. (2009) The value of tumor markers in pulmonary tuberculosis. *Tunis. Med.* **87**, 330–333

118 Zhang, C.X., Zhang, D.J., Wang, Y.L., Han, W., Shi, G.C. and Zhang, H.Q. (2016) Expression level of NSE, S100B and NPY in children with acute miliary pulmonary carcinoma. *Chem. Lab. Med.* **51**, 1493–1498, https://doi.org/10.1515/cclm-2012-0738

119 Rohlwink, U.K., Mauff, K., Wilkinson, K.A. et al. (2017) Biomarkers of cerebral injury and inflammation in pediatric tuberculosis meningitis. *Eur. J. Med. Pharmacol. Sci.* **20**, 1474–1478

120 Nam, S.J., Jeong, J.Y., Jang, T.W. et al. (2016) Neuron-specific enolase as a novel biomarker reflecting tuberculosis activity and treatment response. *Korean J. Intern. Med.* **31**, 694–702, https://doi.org/10.3904/kim.2015.407

121 Barouchos, N., Papazafiropoulou, A., Iacovidou, N. et al. (2015) Comparison of tumor markers and inflammatory biomarkers in chronic obstructive pulmonary disease (COPD) exacerbations. *Scand. J. Clin. Lab. Invest.* **75**, 126–132, https://doi.org/10.3109/03656513.2014.992944

122 Ludtke, T.H., Farin, H.F., Rudat, C. et al. (2013) Tbx2 controls lung growth by direct repression of the cell cycle inhibitor genes Cdkn1a and Cdkn1b. *PLoS Genet.* **9**, e1003189. https://doi.org/10.1371/journal.pgen.1003189

123 Fang, S.C., Lu, K.H., Wang, C.Y., Zhang, H.T. and Zhang, Y.M. (2013) Elevated tumor markers in patients with pulmonary alveolar proteinosis. *J. Cancer Res. Ther.* **9**, 311–320, https://doi.org/10.1378/chest.11-2948

124 Ni, L.F. and Liu, X.M. (2014) Diagnostic value of serum neuron-specific enolase in differentiating malignant from benign solitary pulmonary nodules. *Beijing Da Xue Xue Bao Yi Xue Ban* **36**, 707–710

125 Dong, Y., Zheng, X., Yang, Z. et al. (2016) Serum carcinoembryonic antigen, neuron-specific enolase as biomarkers for diagnosis of non-small cell lung cancer. *J. Cancer Res. Ther.* **12**, 239–253, https://doi.org/10.1515/cclm-2012-0738

126 Aral, T., Inoue, Y., Sugimoto, C. et al. (2014) CYFRA 21-1 as a disease severity marker for autoimmune pulmonary alveolar proteinosis. *Respirology* **19**, 246–252, https://doi.org/10.1111/resp.12210

127 Fang, S.C., Lu, K.H., Wang, C.Y., Zhang, H.T. and Zhang, Y.M. (2013) Elevated tumor markers in patients with pulmonary alveolar proteinosis. *Clin. Chem. Lab. Med.* **51**, 1493–1498, https://doi.org/10.1515/cclm-2012-0738

128 Zhong, W.J., Yang, H.H., Guan, X.X. et al. (2019) Inhibition of glycolysis alleviates lipopolysaccharide-induced acute lung injury in a mouse model. *J. Cell. Physiol.* **234**, 4641–4654, https://doi.org/10.1002/jcp.27261
129 Cui, H., Li, S., Xu, C., Zhang, J., Sun, Z. and Chen, H. (2017) Emodin alleviates severe acute pancreatitis-associated acute lung injury by decreasing pre-B-cell colony-enhancing factor expression and promoting polymorphonuclear neutrophil apoptosis. *Mol. Med. Rep.* **16**, 5121–5128, https://doi.org/10.3892/mmr.2017.7259

130 Manu, K.A., Cao, P.H.A., Chai, T.F., Casey, P.J. and Wang, M. (2019) p21cip1/waf1 coordinate autophagy, proliferation and apoptosis in response to metabolic stress. *Cancers (Basel)** **11**, https://doi.org/10.3390/cancers11081112

131 Guice, K.S., Oldham, K.T., Johnson, K.J., Kunkel, R.G., Morganroth, M.L. and Ward, P.A. (1988) Pancreatitis-induced acute lung injury. An ARDS model. *Ann. Surg.* **208**, 71–77, https://doi.org/10.1097/00000658-198807000-00010

132 Renner, I.G., Savage, III, W.T., Pantoja, J.L. and Renner, V.J. (1985) Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. *Dig. Dis. Sci.* **30**, 1005–1018, https://doi.org/10.1007/BF01308298

133 Owusu, L., Xu, C., Chen, H. et al. (2018) Gamma-enolase predicts lung damage in severe acute pancreatitis-induced acute lung injury. *J. Mol. Histol.* **49**, 347–356, https://doi.org/10.1007/s10735-018-9774-3