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Measurement of Exhaled Markers

Measurement of Nasal Nitric Oxide

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Abstract

The major part of nitric oxide (NO) in exhaled air originates from the nasal airways, with only minor contribution from the lower airways and the oral cavity. The physiological role of the very high local NO concentration in the paranasal sinuses is still unclear. The most widely used and best-standardized method to sample nasal NO in isolation from the lower respiratory tract is aspiration at a fixed flow through the nasal passages in series. Important technical considerations include the choice of the correct transnasal flow and the ability of children to perform a breath-holding manoeuvre. The effects of age and height on nasal NO values have yet to be defined in a larger population of healthy children using the recommended aspiration technique. Presently, there is no validated technique available to measure nasal NO in infants and small children. The measurement of nasal NO concentrations has evoked interest in its potential to serve as a non-invasive and simple diagnostic tool for upper and lower respiratory tract disorders. Measurements of nasal NO concentrations are helpful to screen children with clinical symptoms suggestive of primary ciliary dyskinesia and to exclude this disease in those with high nasal NO concentrations with high certainty. Nasal NO measurements are, however, of no diagnostic utility in distinguishing between other conditions such as asthma, cystic fibrosis, bronchiectasis, sinusitis or rhinitis, or in monitoring therapeutic interventions in any such disorder.

Origin of Nasal Nitric Oxide

Nitric oxide (NO) is produced endogenously within the respiratory tract and was first documented in exhaled air in humans and mammals in 1991 [1]. It was then shown that the major part of NO in exhaled air originates from the nasal airways, with only a minor contribution from the lower airways and the oral cavity [2]. NO is present in the nasal airways and paranasal sinuses in very high concentrations, close to the acute exposure levels set by occupational health guidelines for short-term exposure at the workplace [3, 4].

Biochemical Pathway and Cellular Origin

NO is generated from the semi-essential amino acid *L*-arginine by the enzyme NO synthase (NOS), which can be divided into two major categories: constitutive NOS (cNOS) and inducible NOS (iNOS). The constitutive enzyme, which according to its location may be named endothelial NOS (eNOS) or neuronal NOS (nNOS), is activated by calcium and calmodulin. It produces small amounts of NO to modulate physiological processes, and can be stimulated by bradykinin, acetylcholine, histamine, leukotrienes, and several other mediators. Calmodulin is an enzyme cofactor regulating electron transport. It is also identified in close juxtaposition to the cilia of the upper airway epithelium, and is thought to be involved in ciliary motility. The iNOS, which was first isolated in macrophages, is calcium- and calmodulin-independent and activated by a variety of

pro-inflammatory cytokines and endotoxins. The induction of iNOS requires gene transcription. Hence, an increase in NO production takes hours, but it may continue for days. When activated, iNOS produces up to 1,000 times more NO than cNOS. Corticosteroids only inhibit iNOS [5]. The different NOS are coded by genes on chromosomes 7 (eNOS), 12 (nNOS), and 17 (iNOS). NOS requires oxygen and nicotinamide adenine dinucleotide phosphate as cosubstrates to oxidize *L*-arginine to *L*-citrulline and NO.

Many cells in the upper and lower respiratory system produce cNOS including parasympathetic vasodilator nerves, endothelial cells, and ciliated mucosa cells. iNOS has been reported to be present not only in epithelium but also in macrophages, fibroblasts, neutrophils, endothelium and vascular smooth muscle. The NOS found in abundance in the apical regions of the maxillary sinus epithelium most closely resembles the inducible isoform. It is, however, constantly expressed and not inhibited by steroids which are characteristics commonly associated with cNOS rather than iNOS [3]. It is localized mainly within cilia and microvilli and held responsible for the high NO concentrations within the sinus lumen of healthy humans [3, 6].

Anatomic Origin

The epithelial cells of the paranasal sinuses were identified as a major source of NO production in the respiratory tract. NO concentrations inside the paranasal sinus are several hundred times higher than in exhaled air from lower airways [3,000–25,000 parts per billion (ppb)]. The sinuses communicate with the nasal cavity through their ostia and the rate of gas exchange between these cavities is dependent on several factors such as the size of the ostia, the volume of the sinuses, the nasal airflow, and intranasal pressure. There is still some controversy whether the majority of NO measured in nasal air originates from the paranasal sinuses or from the mucosa of the nasal cavity. The low concentrations of NO found intranasally during an acute sinusitis as well as its increase after antibiotic therapy have preferentially been explained by obstruction and opening of the sinus ostia, respectively [7]. Recent data, however, suggest that iNOS expression is markedly reduced in the sinus epithelium of patients with maxillary sinusitis [6]. Hence, patency of sinus ostia is not the only factor affecting nasal NO concentration during sinusitis. High concentrations of NO are found in the nose of neonates shortly after birth, even before the sinuses have developed [8]. In a unique study, the osteomeatal complex and sphenoidal recess were occluded in one volunteer to isolate the nose from the sinuses. It was shown that, when all the sinus ostia are blocked, nasal NO output is

decreased by only 12%. Interestingly, after ostial occlusion paranasal sinus NO concentration reached a plateau at about 30,000 ppb. This suggests a negative feedback mechanism limiting NO output above a certain local concentration. Further measurements also suggested that, although the NO output per square unit of mucosa was smaller in the nose than in the sinuses, the majority of nasal NO is still derived from the nose itself, because of its larger surface area [9].

Physiological Role of Nasal NO

NO production is commonly enhanced at sites of inflammation. The physiological role of the very high local NO concentration in the paranasal sinuses is still unclear. NO is bacteriostatic at such high concentrations and may contribute to the local host defence of these cavities [3]. It may also play an important role in the regulation of ciliary function [10, 11]. Pulmonary vascular resistance is decreased in humans during nasal breathing compared to that during mouth breathing, intubation or after tracheotomy [12, 13]. This implies that nasally derived NO acts by autoinhalation as an aecrine messenger to modulate the pulmonary vascular tone and to improve ventilation/perfusion matching.

Methodology of Measuring Nasal NO [14]

Many brilliant experimental techniques have been used to measure NO production at the various sites of the upper airways to arrive at the current understanding of nasal NO physiology. The measured NO concentrations differed widely, because they depend highly on the technology and measurement techniques used. Therefore, international task forces have tried to set standards for NO measurements to enable the comparison of results from different laboratories. This chapter will review the currently recommended technique to measure nasal NO concentrations for diagnostic purposes in clinical practice [15, 16].

Terminology and Units

The nasal airway is a complex system of communicating cavities composed of the nasal cavities, paranasal sinuses, the middle ear, and the nasopharynx. Measurements of nasal NO provide no information with respect to the anatomical source of the gas or the physiological processes that generate the NO. The fractional concentration of nasal NO is termed nasal FE_{NO} and expressed in ppb, which is equivalent to nanolitres per litre. Nasal NO output represents the amount of nasal NO exhaled per time unit, and is denoted nasal \dot{V}_{NO} . It is calculated from the product of NO

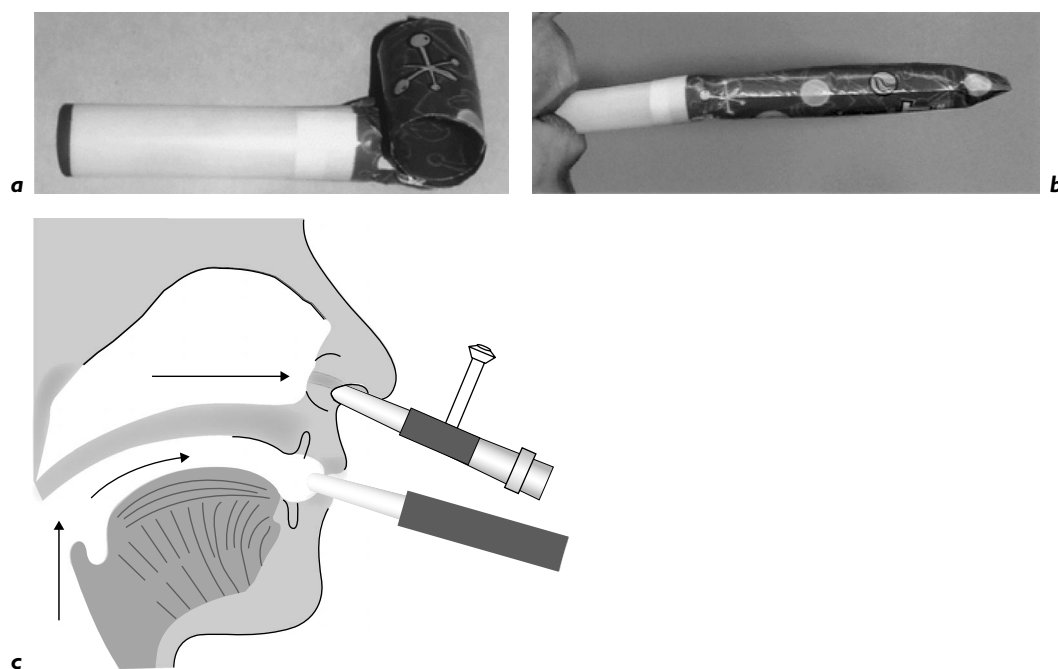


Fig. 1. **a** Party toy. **b** Party toy inflated. **c** Velopharyngeal closure achieved with inflation of party toy [reproduced with permission from 21].

concentration in nanolitres per litre and expiratory flow rate in litres per minute, corrected to BTPS.

NO Analyzer

NO is measured in exhaled air by chemiluminescence which is based on the emission of light from the reaction of NO with ozone (O_3) to NO_2 . The quantity of light emitted is proportional to the concentration of NO [17]. The extremely high sensitivity and fast response time of modern NO analyzers permit continuous on-line measurements of NO in exhaled air. Minimum standards for suitable chemiluminescence NO analyzers have been described in detail with respect to linearity and accuracy ($\pm 1\%$ full range), lower detectable limit (≤ 1 ppb), response time (fast lag time and rise time), and measurement range (0.1–10,000 ppb) [15].

Description of Standard Technique

Measurement of nasal NO output requires generation of air flow through the nasal cavity (transnasal air flow). While the velum is closed, transnasal flow can be achieved by various aspiration or insufflation methods which generate flow through the nasal cavities in series (air circulates from one naris to the other) or in parallel. Aspiration at a fixed flow through the nasal passages in series is currently

the most widely used and best-validated method to sample nasal NO in isolation from the lower respiratory tract.

Velum Closure

Measurements have to be performed during velum closure to exclude air entry from the lower respiratory tract and to prevent a loss of nasal air via the posterior velopharyngeal aperture. Recommended methods to achieve velum closure are: (1) inhalation to total lung capacity and exhalation against an expiratory resistance while targeting a mouth pressure of 10 cm H_2O [18], (2) breath holding [19], (3) pursed lip breathing [20], and (4) sustained inflation of a party toy [21].

Slow oral exhalation against a resistance of at least 10 cm H_2O has been chosen as the preferred method in adults, but any method that can reliably close the velum is acceptable. It is our experience and the experience of others that in older children velum closure can be reliably achieved by breath holding or exhalation into a balloon or 'party toys' which can act as expiratory resistors (fig. 1) [21, 22]. The children are asked to keep the party toy inflated until a maximum plateau NO concentration is reached. The sustained inflation of the party toy indicates that palatal closure is reliably achieved. Simultaneous measurement of nasal CO_2 is used

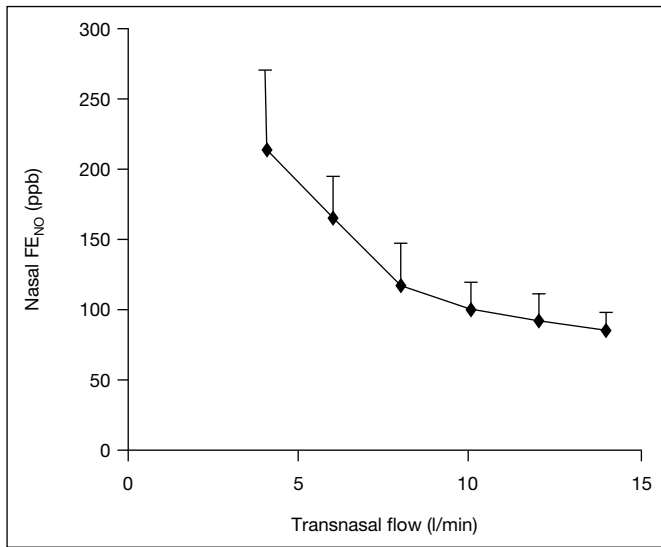


Fig. 2. Inverse correlation of nasal NO concentration (FE_{NO}) with transnasal flow rate [reproduced with permission from 16].

to verify velum closure and to demonstrate the absence of contamination with exhaled air from the lower airways. There is, however, no validated technique to measure nasal NO concentrations in infants and young children who are unable to perform velum closure manoeuvres.

Aspiration or Sampling Flow

It is essential to measure the NO concentration at a known and fixed aspiration flow, because nasal NO concentration is inversely related to transnasal flow rate (fig. 2) [23]. The transnasal or aspiration flow should be measured and recorded against time together with the NO concentration and CO_2 level. The constant transnasal flow produces a washout phase of NO followed by the establishment of a steady plateau documented in the profile of NO (fig. 3). It has been demonstrated that nasal NO output varies with the magnitude of aspiration flow, despite the achievement of a steady-state plateau of NO concentration in the aspirated air. NO output is higher when measured at transnasal flows in the magnitude of 2.7–3.7 l/min compared with lower flows of 0.2–0.7 l/min. The higher flows produce a turbulent instead of a laminar flow pattern and facilitate ventilation of the narrow peripheral parts of the nasal airway. It is also argued that the efficacy of NO removal from the nasal mucosa is higher with turbulent flow, as is water and heat transfer. These turbulent flows also most closely resemble physiological flows during quiet nasal breathing.

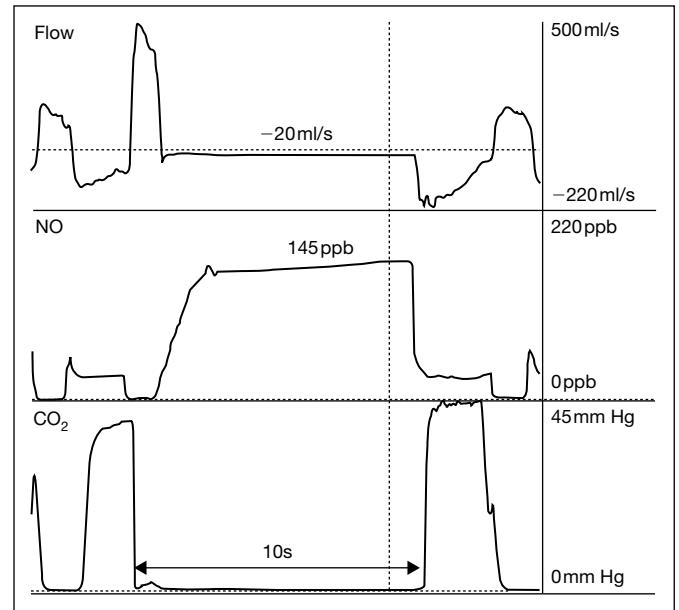


Fig. 3. Simultaneous measurements of flow, NO concentration and CO_2 level against time. The NO trace shows a washout phase and steady plateau at a fixed transnasal flow of 20 ml/s. The CO_2 signal confirms the absence of contamination with alveolar air.

Hence, NO measurements obtained at low laminar flows may underestimate NO output compared to measurements at higher and more physiological transnasal flow rates. By reporting the NO output instead of the NO concentration, measurements at different flows become comparable, provided the aspiration flow is within the flow range that provides maximal and stable NO concentrations (0.9–6.0 l/min) [24]. An ATS task force defined 3.0 l/min as the optimal flow to measure nasal NO concentration in adults [16]. However, no specific recommendations were made for optimal transnasal flows in children, which are likely to be lower. The optimal range of flows was later reported to be 3.2–5.2 l/min in adults and 2.2–3.2 l/min in children [25]. We obtained steady plateau NO concentrations in children with transnasal flows at 1.2 l/min [22]. It is recommended to increase aspiration flow rates up to 6 l/min, if a steady NO plateau is not achieved. The precise flow used should be documented for each subject.

Technique

Nasal NO is measured in the child sitting with an olive introduced approximately 1 cm inside one nostril ensuring a tight seal while the contralateral nostril is left open



Fig. 4. Measurements of nasal NO output during breath hold (**a**) and during constant insufflation into an expiratory resistor (**b**). Aspiration flow is measured by an ultrasonic spirometer connected to a vacuum source and CO₂ by capnography, respectively.

(eventually with the help of another nozzle). This olive should be composed of a soft, non-traumatizing material, and be of sufficient diameter and appropriate shape to occlude the naris. Air is aspirated at a constant flow rate by a suction pump while the velum is closed by an appropriate technique for at least 10 s. A side port just distal to the olive samples gas for NO analysis (fig. 4). A tight-fitting mask covering the nose may also be used for nasal NO measurements.

Humming and Assessment of Sinus Ventilation

Nasal NO concentrations increase largely (15-fold) during humming compared to silent exhalation [26]. This is explained by an increased washout of air, and hence NO, from the paranasal sinuses into the nasal cavity by the oscillating sound waves. The increase in nasal NO during humming is absent if the sinus ostia are completely obstructed. Hence, combined nasal NO measurements with and without humming could be of use to estimate sinus ventilation and to assess the relative contribution of the nasal cavity and the sinuses to nasal NO output [27]. Nasal NO is markedly decreased following repeated consecutive humming manoeuvres and recovers to baseline concentrations after a 3-min period of silence. This pattern fits well with the notion that humming empties the sinuses and that a period of silence will allow for NO to accumulate again. It is of interest that posthumming nasal NO measurements are characterized by less intrasubject variability in comparison with measurements performed after a short period of speaking or silence [28]. Posthumming measurements may serve as a measure of NO output from the nasal cavity mucosa.

Factors Influencing Nasal NO Values

There is only limited knowledge on whether and how physiological, pharmacological, and external factors affect nasal NO output. It is evident that breath holding or nose occlusion increase nasal NO measurements.

Ambient Air

Ambient NO concentrations are highly variable and can reach concentrations that may cause considerable errors if ambient air is used as the gas source for transnasal flow. This is of utmost concern if ambient NO concentrations are higher than nasal NO concentrations, e.g. in conditions with very low nasal NO output such as primary ciliary dyskinesia (PCD). The use of NO-free air as gas source for transnasal flow is recommended to eliminate this problem. In any case, the ambient NO concentration should be recorded and taken into consideration for correct interpretation of nasal NO measurements.

Smoking

The effect of prenatal or postnatal tobacco smoke exposure on nasal NO concentration has not been investigated in children. Nasal NO concentrations are slightly lower in smokers [29].

Age

It has been hypothesized that nasal NO concentrations rise from birth until the age of 10 years with pneumatization of the sinuses, although when related to body weight NO output in preterm infants was shown to be similar to

Table 1. Published studies measuring nasal NO in healthy children

Study	n	Age years	Technique	Aspiration flow, l/min	No output, nl/min	NO concentration, ppb
Corbelli et al. [22]	24	12.4 (4.5–24)	Breath hold	1.2		223.7 (90–950)
Daya et al. [21]	30	10.7 (3.3–17.5)	Breath hold	3	458 (131–1,424)	
Narang et al. [48]	53	10.7 (5.5–19)	Breath hold	0.25		716 (398–1,437)
Karadag et al. [36]	20	~10.8	Breath hold	0.25		553 (116–1,437)
Baraldi et al. [38]	133	6–15	Tidal breathing	0.7		216 (95% CI 204–228)
Balfour-Lynn et al. [37]	54	12.2 (6–17)	Breath hold	0.25		1,024 (158–2,502)
Lundberg et al. [40]	19	5–15	Tidal breathing	0.7		239 (SD 20)
Dötsch et al. [46]	37	4–18	Tidal breathing	0.7		101 (SD 49)

Data represent mean or median with the range in parentheses except where stated otherwise. CI = Confidence interval; SD = standard deviation.

adults [30]. More work is needed to clarify age-related changes in nasal NO output from infancy to adulthood using comparable methods. Nasal NO concentrations in adults are not affected by aging.

Physical Exercise

Nasal NO concentrations decrease by about 50% during physical exercise and reach normal baseline concentrations in about 15–20 min [31, 32]. The reason for the decrease in nasal NO output during exercise has not yet been elucidated.

Drugs

Data on the effect of pharmacological substances on nasal NO output is limited. Nasal decongestants, such as oxymetazoline and xylometazoline, decrease nasal NO concentrations by about 15% and have a dose-dependent inhibitory effect on total iNOS activity in vitro [33, 34]. Histamine, topical and systemic steroids, and antibiotics have no effect on nasal NO concentrations in healthy persons [35].

Normal Data of Nasal NO Measurements in Children

Data obtained from healthy children demonstrate considerable intersubject variation resulting in a broad range of normal nasal NO output [21]. Normal values have yet to be established in a larger population of healthy children of different ages using the recommended aspiration technique

Table 2. Effect of diseases on nasal NO concentrations in children

Disease	Nasal NO concentration
Common cold, upper respiratory tract infection	Unchanged
Allergic rhinitis	Increased (decreased with topical steroids)
Asthma	Unchanged
Acute sinusitis	Decreased
Cystic fibrosis	Decreased
Non-cystic fibrosis or non-PCD bronchiectasis	Decreased
PCD	Extremely low

during breath holding. Previous studies reporting nasal NO concentrations in healthy children are summarized in table 1. Intrasubject coefficients of variation of repeated measurements by the aspiration technique are between 4 and 8% in healthy children [36–38].

Diagnostic Use in Paediatric Respiratory Diseases

Nasal NO concentrations are increased in asthma, allergic rhinitis, and viral respiratory infections, but are reduced in sinusitis, cystic fibrosis, PCD, bronchiectasis, chronic cough, diffuse panbronchiolitis, and after exposure to tobacco and alcohol (table 2) [37, 39–46]. The most

Table 3. Published studies reporting sensitivity and specificity of nasal NO concentrations in discriminating between patients with PCD and non-PCD bronchiectasis

Study	Aspiration flow, l/min	Cutoff level, ppb	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
Horvath et al. [51]	0.25	187	93	95	87	97
Narang et al. [48]	0.25	250	97	90	83	97
Corbelli et al. [22]	1.2	105	94	88	89	94

comprehensive and significant changes in nasal NO concentrations in relation to normal values can be documented in patients with PCD. Current knowledge suggests that the measurement of nasal NO concentrations may be of clinical value in clarifying diagnostic problems in patients with clinical suspicion of PCD. Nasal NO measurements are, however, of no diagnostic utility in distinguishing between conditions such as asthma, cystic fibrosis, bronchiectasis, sinusitis or rhinitis, or in monitoring therapeutic interventions in any such disorder.

Primary Ciliary Dyskinesia

PCD constitutes a recessively inherited group of disorders of ciliary structure and/or function resulting in impaired mucociliary clearance. Typical structural ciliary abnormalities include absent inner or outer dynein arms, and radial spoke and tubular defects. The clinical manifestations are recurrent or chronic respiratory tract infections with mucus retention as there are rhinitis, sinusitis, serous otitis media, and bronchitis. Mirror image arrangement occurs in 50% of the patients (Kartagener syndrome) [47].

Measurements of nasal NO concentrations in patients with PCD are extremely low compared to healthy children or children with other respiratory disorders [22, 36, 48–52]. There is no relationship between the different

structural defects in PCD and the levels of nasal NO concentration [52]. The reason for the low nasal NO concentrations in patients with PCD has not yet been clarified. Several observations suggest that NO plays an important role in signal transduction associated with ciliary motility. The epithelial NOS is localized at the basal body of the microtubules of the cilia, and NO has been found to stimulate ciliary beat frequency [6, 53]. It seems, however, unlikely that the lower levels of nasal and also exhaled NO concentrations in PCD are the results of reduced NOS activity, because levels of NO metabolites are not different between patients with PCD and healthy subjects [54].

Measurements of nasal NO concentrations are helpful to screen children with clinical symptoms suggestive of PCD and to decide on the need for further, more invasive testing. This is strengthened by the high sensitivity and specificity of nasal NO concentrations to discriminate between PCD and other disorders with chronic airway inflammation, such as cystic fibrosis and non-PCD bronchiectasis (table 3). If nasal NO concentration is unexpectedly low in a patient with recurrent respiratory infections, the diagnosis of PCD should be actively excluded. This is done by assessing the ciliary beat frequency by light microscopy, and searching for the typical ultrastructural defects of the cilia by electron microscopy in mucosal biopsy specimens.

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