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EDITORIAL

Challenges facing early detection of acute kidney injury in the critically ill

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Abstract

Recent advances in the detection of acute kidney injury (AKI) afford the possibility of early intervention. Proteomics and genomics have identified many markers of tubular cell injury, some of which are manifest in the urine. One trial has used novel injury biomarkers to recruit patients to an intervention prior to an elevation in plasma creatinine. This trial and other recent studies have shown that the use of biomarkers of injury will depend on the time the patient presents following insult to the kidney, the likely cause of that insult, and the pre-injury renal function of that patient. The definition of AKI is likely to change in the near future to include a measure of injury. We anticipate novel therapies becoming available following successful trials that utilize the methodology of early intervention following an elevated injury biomarker.

Key words: Acute kidney injury; Acute renal failure; Biomarkers; Creatinine

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INTRODUCTION

Critical care medicine has responded impressively over the last few decades to the challenges of acute lung injury, cardiac arrest, and sepsis, but not so well to the challenge of acute kidney injury (AKI). Recent consensus definitions have helped establish the incidence to be about 35% to 70% in the intensive care unit (ICU)[1,2], yet other than renal replacement therapy (RRT) there are no established treatments.

AKI has several etiologies including renal ischemia, nephrotoxic injury, and AKI complicating sepsis, which complicate detection and treatment. Almost inevitably diagnosis is only at the late stages of the disease, or retrospectively. This is because of the reliance on serum creatinine as a marker of glomerular filtration rate (GFR).

Creatinine is formed from creatine in the muscles, at a constant rate, and with a molecular weight of 113 Da is freely filtered at the glomerulus. When kidney function is normal the rate of production of creatinine is matched by the rate of renal excretion. If GFR decreases, plasma creatinine slowly increases to a new steady state con-

centration that reflects the new GFR. At normal GFR the half-life of creatinine is about 4 h. This increases as GFR decreases, thus only after 24-72 h (3 to 5 half lives with lower GFR) will a new steady state concentration be reached. The current consensus definition of AKI [risk, injury, failure, loss, end-stage (RIFLE)[3]], of AKI requires at least a 50% increase in plasma creatinine. This results from at least a 33.3% rapid decline in GFR[4]. The delay in a measurable increase in plasma creatinine is further exacerbated by accuracy of plasma creatinine measurements; nephrologists typically look for changes of at least 10% before accepting these. Additionally, the practice of fluid administration in the ICU dilutes creatinine concentration[5]. Figure 1 illustrates a delayed increase in plasma creatinine following a decrease in GFR (estimated by a 4-h creatinine clearance) of a patient from the EARLYARF trial[6].

Inevitably, delayed diagnosis means delayed treatment. In the case of AKI the clinician's options are limited. They may minimize harm by withdrawing nephrotoxins, and may attempt to replace or increase circulating volume by fluid-loading, or, in worst case, initiate RRT. Recent evidence suggests that fluid-loading (rather than a neutral fluid-balance) may be detrimental rather than supportive, potentially reducing options further[7]. Delayed diagnosis may also contribute to the failure of many interventions that were promising under experimental conditions in animals[8].

Recent advances in proteomics and genomics have breathed new life into the quest for successful treatment of AKI. The search for specific and sensitive injury biomarkers has become a global focus of the nephrology and critical care communities. The paradigm is quite simple; following insult to the kidney a molecule is released into the urine or plasma where it is detected and treatment initiated before, or soon after a decrease in GFR. There has been considerable success in identifying candidate biomarkers[9-12]. In this review we explore whether these new biomarkers will supplant or merely support creatinine in the ICU.

The patient undergoes an immediate decline in GFR (4-h-creatinine clearance; open circles) of 38% in 24 h. The increase in plasma creatinine (closed circles) is not detectable until 48 h and peaks as a 46% increase only at 72 h. The subsequent decline in plasma creatinine suggestive of recovery is also delayed compared with the increase in creatinine clearance 24 h earlier. Error bars are ± 10% indicating measurement uncertainty (Figure 1).

THE EARLY INTERVENTION PARADIGM

The EARLYARF study was the first trial to employ kidney injury biomarkers of AKI to recruit patients to the intervention arm of a randomized control trial of a novel intervention[6]. Described as a "glimpse of the future"[13], this trial illustrates the challenges faced by this new paradigm.

On entry to the ICU, at 12, 24 h, and then daily for 7 d

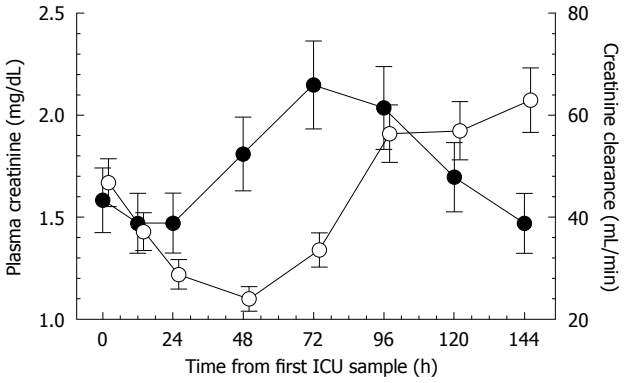

Figure 1 Delayed diagnosis. ICU: Intensive care unit.

the urine of at risk patients were monitored for elevated concentrations of the brush border enzymes alkaline phosphatase (AP) and γ-glutamyl-transpeptidase (GGT). These biomarkers were chosen on the basis of a pilot study which had shown them to be highly sensitive and specific for AKI[10] and because they could be measured in a hospital diagnostic lab with rapid turnaround. Since the inception of the EARLYARF trial in 2005 a number of other biomarkers, discussed below, have proven to hold out greater promise as early injury biomarkers, some of which have now entered commercial production and could be used for future early intervention trials. AP and GGT were normalized to urinary creatinine concentration to account for variation in water reabsorption and, in order to avoid false positives, the product GGT × AP > 46.3 was used to recruit patients to an intervention of either two doses of high dose erythropoietin (500 U/kg) or placebo (normal saline) 24 h apart. Erythropoietin was chosen for its anti-apoptotic property and following success in animal studies in ischaemic/repurfusion injury[14,15]. The primary outcome was the difference in the mean relative average value of creatinine (RAVC) of the two groups. The RAVC is the average plasma creatinine increase from baseline as a percentage of baseline creatinine[16]. The difference in the mean RAVC between control and treatment groups is more sensitive to small differences in renal function than a categorical marker such as RIFLE[16,17].

Lessons on use of early biomarkers

The EARLYARF trial did not show Erythropoietin to be an effective early intervention in AKI, however, it did not preclude this possibility. This is because of the limited utility shown by GGT × AP as a recruitment tool. Whilst GGT × AP > 46.3 did select patients with more severe illness and at greater risk of AKI, needing RRT, and death from the general ICU population there was still a considerable risk of AKI in those not triaged. Analysis of the time profile of GGT × AP taken from a putative time of insult (determined retrospectively) showed that GGT × AP is most likely to be elevated in the first 12 h following insult. For many patients entering the ICU the putative time of insult was more than 12 h earlier, par-

ticularly in the case of progressive diseases such as sepsis. An analysis of the timing of the first dose of the study drug showed that it was administered a median 12.9 h following putative insult, outside the experimentally determined optimal treatment window of within 6 h of injury for erythropoietin[14]. Thus, the first lesson is that injury biomarkers have a temporal window of opportunity following injury in which they are diagnostic. If the time from insult is unknown a negative biomarker is not necessarily indicative of no-injury or no change in function. Given the relative short duration of elevation of some of these biomarkers the second lesson is that repeated measures of biomarkers about 3-6 h apart will be necessary to avoid false negatives by missing the temporal window of opportunity.

In addition to AP and GGT four other urinary injury biomarkers were measured, namely: kidney injury molecule-1, neutrophil-gelatinase-associated-lipocalin (NGAL), interleukin-18 and cystatin C (CysC). Each demonstrated a unique temporal profile[18]. Furthermore, as had been demonstrated with NGAL[19], the diagnostic performance was shown to depend on the underlying baseline (normal) renal function. Optimal diagnostic ability for each biomarker depended on the combination of both time from insult and pre-injury renal function. For example, CysC was diagnostic of AKI when measured 6 to 12 h from insult in those with estimated baseline GFR (eGFR) of 90 to 120 mL/min with an area under the receiver operator curve, AUC, of 0.89 (95% CI: 0.70-1), but was not diagnostic of those with lower eGFR during the same time period. The third lesson is that biomarkers must be chosen according to each patient's pre-injury renal function.

There have been a proliferation of studies identifying potential AKI biomarkers in addition to those already described, including liver-fatty acid binding protein[20], albumin[21], netrin[22], α- and π-glutathione-S-transferase[10,23], and β2-microglobulin[24]. There are several recent reviews which cover the potential of several biomarkers to be early markers of AKI and describe their pathophysiology[11,12,25,26]. The most studied of biomarkers is plasma and urinary NGAL. A meta-analysis of 19 clinical studies involving more than 2500 patients resulted in an overall AUC of 0.82 (0.73-0.89) for diagnosis of AKI. The AUC in critically ill patients was lower, 0.73 (0.62-0.83)[27]. The AUC for prediction of RRT was 0.78 (0.65-0.92). This performance is good without being spectacular, however, as the authors report, it is similar to the AUC range for troponin detection of myocardial infraction during its clinical implementation.

AKI injury biomarkers have been assessed almost exclusively on the basis of their ability to detect or predict a rise in plasma creatinine. This injury-function method is potentially misleading. It assumes that a change in function that results in an observable change in plasma creatinine is more important than an increase in injury biomarkers *per-se*. This remains to be seen. The subcategory of biomarker positive/creatinine negative patients has received scant attention. Only one study has addressed this directly. Haase *et al*[28] analysed plasma and urinary NGAL data from 10 studies and concluded that NGAL-positive/creatinine-negative patients were more likely to require RRT, more likely to die in hospital and had longer lengths of ICU and hospital stay than NGAL-negative/creatinine-negative patients. This illustrates the potential for an injury biomarker to stand alone from creatinine as a marker of AKI. In this study patients with both an elevated NGAL and elevated creatinine were more likely to require RRT or die in hospital than those with only biomarker elevated. The fourth lesson is that future diagnosis will involve biomarkers of both injury and function. Before this goal can be realized appropriate cutpoints for the various AKI injury biomarkers must be determined.

Injury biomarkers may loosely be classified as preformed, such as brush border-enzymes AP and GGT, or induced (upregulated) through some injury mechanism, such as KIM-1 from tubular epithelial cells during the process of dedifferentiation and re-proliferation[29,30]. Biomarkers pre-formed in the plasma (e.g., CysC and albumin) or absorbed into the plasma following tubular injury (e.g., NGAL) may also be present in the urine due to failure of the tubular transport mechanisms to reabsorb them from the tubular fluid[31]. Potentially, an improved understanding of disease pathways may lead to utilization of biomarkers according to suspected cause of injury rather than the one biomarker fits all approach of the EARLYARF trial. The recent work of the Predictive Safety Testing Consortium on pre-clinical nephrotoxic biomarkers is revealing in this respect, as not all biomarkers responded to all nephrotoxins. For example, urinary CysC and β2-microglobulin were elevated for Purcomycin and Doxorubin but not Cisplatin or Gentamicin whereas Clusterin was elevated for all these toxins[32]. The fifth lesson is that a panel of biomarkers that respond to the range of possible different AKI causes of patients entering intensive care will be required to capture all cases.

We have argued that the removal of a change in GFR (leaving only a change in the surrogate plasma creatinine) from the AKIN consensus definition of AKI was a mistake[33,34]. Short duration creatinine clearance potentially provides an earlier indicator of GFR than plasma creatinine (Figure 1) and new technologies are under development which may lead to a direct, near real-time, measure of GFR. We have reviewed these recently[30]. For example, a device attached to a patient's arm, the ambulatory renal monitor (ARM) measures the decay of ^{99m}Tc-DTPA for up to 24 h following a single injection. A change in GFR could be detected within 5-10 min[35,36]. A second technique involves two fluorescent markers, one cleared by the kidney and one not. The ratio between these markers when observed with imaging of blood vessels in the skin of rats provided a measure of GFR[37,38]. The sixth lesson is that rapid measures of GFR may provide an adjunct to injury biomarkers for the early detection of AKI.

Table 1 Possible future modes of diagnosing acute kidney injury

AKI diagnosis	Timing	Staging	Examples
Injury biomarkers	Within 0-12 h of injury and every 3 to 6 h	Cutpoints of concentrations	Urinary NGAL, cystain C, IL-18, KIM-1, GGT, L-FABP, Plasma NGAL
Functional measurements	Any time following injury	Change from a baseline	4 h creatinine clearance, ARM urine output
Surrogates of function	12 h to 7 d post injury	Change from a baseline	Plasma creatinine, plasma cystatin C

NGAL: Neutrophil-gelatinase-associated-lipocalin; IL-18: Interleukin-18; KIM-1: Kidney injury molecule-1; GGT: γ-glutamyl-transpeptidase; L-FABP: Liver-fatty acid binding protein; ARM: Ambulatory renal monitor; AKI: Acute kidney injury.

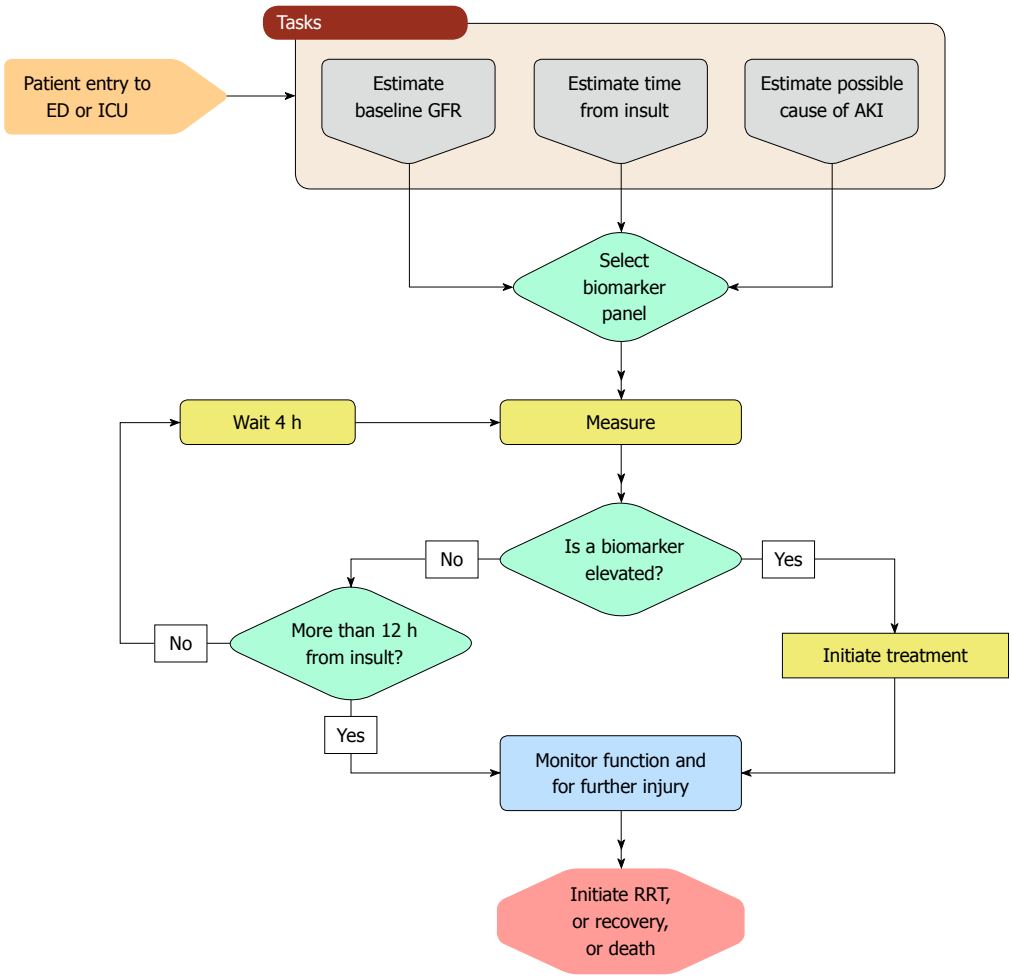

Figure 2 Potential algorithm for utilizing an acute kidney injury injury biomarker on arrival in the emergency or intensive care unit. ICU: Intensive care unit; RRT: Renal replacement therapy; GFR: Glomerular filtration rate; AKI: Acute kidney injury.

Plasma CysC has been proposed as an alternative surrogate of GFR to plasma creatinine. CysC is a low molecular weight protein, 13.3 kDa, produced at a constant rate and freely filtered through the glomerulus[39]. Unlike creatinine it is reabsorbed in the proximal tubule by megalin-facilitated endocytosis[31]. CysC has one-third the volume of distribution of creatinine meaning that any loss of renal function will be reflected by a more rapid rise in plasma CysC than plasma creatinine. Herget-Rosenthal *et al*[40] first demonstrated the potential of plasma CysC in the ICU. In a group of 44 patients the mean time to an increase of 50% in CysC was 1.5 ± 0.6 d earlier than the mean time to an increase of 50% in creatinine. In the larger EARLYARF trial the difference in time for each individual was calculated resulting in a mean difference of 5.8 ± 13 h[41]. While these differences appear modest, sampling was still 12 to 24 h apart and investigations with more frequent measurement will determine if CysC may detect loss of GFR on shorter time scales. Because it is likely to respond to changes in GFR more quickly than creatinine, sampling of CysC is likely to need to be more frequent to observe these changes. CysC also has the advantage that it is less influenced by muscle mass (and hence, sex and age) than plasma creatinine, however it is influenced by thyroid dysfunction, some cancers and glucocorticoids[42-44]. The assumption of a constant

production rate of CysC has yet to be investigated in the critically ill. Any switch to CysC would involve considerable expense, partly because of the more complex assay methods and also because to be effective it would need to become widely measured outside of the ICU as well. The final lesson is that plasma CysC has the potential to supplant creatinine as a surrogate measure of renal function, although there is insufficient evidence to justify the expenditure of replacing creatinine.

FUTURE PERSPECTIVE

AKI will be diagnosed using multiple definitions incorporating injury biomarkers, functional measurement, and surrogates of function (Table 1). The earliest diagnosis will be by either an elevated injury biomarker or a change in a functional measure of GFR. Injury biomarkers will be measured on arrival in an emergency department of ICU. One elevated biomarker (e.g., 2 times above the normal upper limit[45]) may be sufficient to diagnose AKI. However, the absence of an elevated injury biomarker would not exclude the possibility of AKI because of the possibility that the time between the injury and the measurement is outside of the window of opportunity for that biomarker. As with the use of troponins to monitor for myocardial infarction, repeated measurements of AKI injury biomarkers a few hours apart will be necessary. A measurement of function, either through a brief creatinine clearance, possible now, or a device such as the ARM monitor in the future, may be used to aid diagnosis. However, definitive diagnosis will only be possible if a baseline GFR has been measured (e.g., prior to surgery) or can be estimated with reasonable accuracy. For many critically ill patients the value of monitoring function will be as an early indication of hospital acquired AKI and will continue to be important for deciding when to initiate RRT. Urine output will continue to be used, although recent evidence suggests that it is often associated with physiological changes other than AKI[46]. Surrogate markers of function, particularly creatinine, which are the current primary tool for diagnosing AKI will still have a place, but because they are a very much later diagnosis their value will be limited to when the window for the particular injury biomarkers has been missed, and when the first plasma surrogate measurement is within the normal range. Increases in the plasma surrogate would suggest an earlier loss of function. Figure 2 is a possible algorithm for implementation of injury biomarker of AKI in the ICU.

CONCLUSION

Novel injury biomarkers will soon be incorporated into the definition of AKI alongside current surrogates of renal function. These biomarkers will change practice in the ICU once efficacious early intervention treatments are discovered. These will require randomized control trials which utilize the trial methodology of the EARLYARF trial, namely recruitment following elevation of an injury biomarker. Plasma creatinine will continue to play a role as a functional marker until replaced by a more responsive functional marker such as plasma CysC or a rapid measure of GFR.

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TOPIC HIGHLIGHT

Ayman Abd Al-maksoud Yousef, MD, *Series Editor*

Prognostic categorization of intensive care septic patients

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Abstract

Sepsis is one of the leading worldwide causes of morbidity and mortality in critically-ill patients. Prediction of outcome in patients with sepsis requires repeated clinical interpretation of the patients' conditions, clinical assessment of tissue hypoxia and the use of severity scoring systems, because the prognostic categorization accuracy of severity scoring indices alone, is relatively poor. Generally, such categorization depends on the severity of the septic state, ranging from systemic inflammatory response to septic shock. Now, there is no gold standard for the clinical assessment of tissue hypoxia which can be achieved by both global and regional oxygen extractabilities, added to prognostic pro-inflammatory mediators. Because the technology used to identify the genetic make-up of the human being is rapidly advancing, the structure of 30 000 genes which make-up the human DNA bank is now known. This would allow easy prognostic categorization of critically-ill patients including those suffering from sepsis. The present review spots lights on the main severity scoring systems used for outcome prediction in septic patients. For morbidity prediction, it discusses the Multiple Organ Dysfunction score, the sequential organ failure assessment score, and the logistic organ dysfunction score. For mortality/survival prediction, it discusses the Acute Physiology and Chronic Health Evaluation scores, the Therapeutic Intervention Scoring System, the Simplified acute physiology score and the Mortality Probability Models. An ideal severity scoring system for prognostic categorization of patients with systemic sepsis is far from being reached. Scoring systems should be used with repeated clinical interpretation of the patients' conditions, and the assessment of tissue hypoxia in order to attain satisfactory discriminative performance and calibration power.

Key words: Prognostic markers; Genome; Sepsis; Systemic inflammatory Response syndrome; Severity scoring systems

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INTRODUCTION

Prognostic categorization of the intensive care unit (ICU) patients with systemic sepsis may be tried through sequential clinical interpretations, assessment of tissue hypoxia and the use of severity scoring systems. The major prognostic value of scoring systems is mainly to compare the effectiveness of ICU services in different centers or over time. So, to determine patient outcome both the clinical interpretation of patients, the assessment of tissue hypoxia, and the scoring systems are together needed.

Table 1 Mortality in various degrees of severity of sepsis

Diagnosis	Number (1100)	Deaths (%)
Nil	421	101 (24.0)
SIRS	573	152 (26.5)
Sepsis	50	18 (36.0)
Severe sepsis	23	12 (52.2)
Septic shock	33	27 (81.8)

SIRS: Systemic inflammatory response syndrome.

In 1991[1], experts from a variety of disciplines met for a Consensus Conference and proposed definitions for sepsis as follows: systemic inflammatory response syndrome (SIRS): It denotes the systemic inflammatory response to a wide variety of severe critical insults, manifested by two or more of the following conditions: temperature > 38 ℃ or < 36 ℃, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mmHg, and white blood counts > 12 000/mL, < 4/L, or > 10% immature forms. Sepsis: It denotes the systemic inflammatory response to infection. Severe Sepsis: It denotes sepsis or SIRS associated with organ dysfunction, hypoperfusion or hypotension.

Hypotension and hypoperfusion abnormalities may include lactic acidosis, oliguria or acute alteration in mental status. Systolic blood pressure < 90 mmHg or a reduction of 40 mmHg from the baseline in the absence of other causes of hypotension notify severe sepsis or SIRS. It is usually corrected by fluid loading. Septic Shock: It denotes sepsis or SIRS induced hypotension not corrected by fluid loading and needing inotropic and/or vasopressor support. Perfusion abnormalities to many organs characterize the shock state. Multiple organ dysfunction (MOD) Syndrome**:** It represents altered organ functions in an acutely-ill patient to the extent that homeostasis cannot be maintained without intervention.

It has been shown that the systemic inflammatory response to severe infection evolves in stages, from sepsis to severe sepsis to septic shock, with corresponding increase in the proportion of patients with positive blood cultures, end-organ failure, and crude mortality[2].

Severe sepsis and septic shock are major reasons for ICU admission. In critically-ill patients in the ICU, who are already compromised because of co-existing serious co-morbidities, septic shock may be associated with higher mortality[3]. In septic patients, the number of organ systems with impaired function is important because it correlates with clinical patient outcome[4].

Sepsis is one of the leading causes of morbidity and mortality worldwide today. It is estimated that there are approximately 700 000 cases of severe sepsis annually in USA and around 400 000 patients die every year as a result of sepsis in both USA and Europe. The incidence of the various degrees of severity of sepsis is not well known but a relatively small Italian study which looked at 1100 ICU admissions as early as 2001, found the following[5] (Table 1):

CLINICAL ASSESSMENT OF TISSUE HYPOXIA IN SEPSIS

Tissue hypoxia is defined as a decrease in the partial pressure of oxygen in a given tissue or as a condition in which the cells of a tissue have abnormal oxygen utilization such that the tissue is experiencing anaerobic metabolism.

Global oxygen consumption/body oxygen delivery relationship

The relationship between whole body oxygen delivery (DO_2) and oxygen consumption (VO_2) in human sepsis has been extensively studied but remains controversial. The pathological supply dependency is an evidence of occult tissue hypoxia and has been associated with an increased incidence of MODS and poor outcomes in patients with sepsis[6]. Support for this belief comes from some clinical investigators who have demonstrated improved outcomes in patients with septic shock by pharmacologically augmenting systemic oxygenation to supranormal levels[7,8]. However, other investigators thought that these clinical studies should be criticized because of methodological error from mathematical coupling because DO_2 and VO_2 were calculated from a common set of measured variables; cardiac output and arterial oxygen content[9]. The author of the present review could not report significant reduction in mortality in septic patients managed by using the supra-normal hemodynamic approach[10].

However, it may be prudent to think that indices of supra-normal oxygenation for management of patients with sepsis may be used for their prognostic categorization. Patients who can attain supra-normal values have decreased morbidity and mortality, mostly due to better physiological reserves. Based on this, it may be concluded that global VO_2/DO_2 relationship based on good oxygen extractability potentiality may denote that the oxygen extraction ratio is an excellent parameter for prognostic categorization of patients with sepsis.

Mixed venous oxygen saturation (SvO_2) determination by pulmonary artery catheterization is a flow-weighted average of venous effluent from all perfused vascular beds. A decrease in SvO_2 can be caused by a decrease in DO_2 and/or an increase in VO_2. An increased value in septic patients denotes tissue hypoxia and its improvement by normal or supra-normal pharmacological interventional therapy may be used as a good prognostic marker[7].

Metabolic lactic acidosis development is one of the most important abnormalities of tissue hypoxia due to the production of hypoxic global lactate during sepsis or septic shock. Plasma lactate has been shown to be a good prognostic indicator of hypo-perfusion in critically-ill patients. Plasma lactate is easy to measure, and lactate clearance can be followed sequentially to assess the prognosis of the response of septic patients to therapy. The more the decrease in pH and the higher the value

of base deficit, the more serious the condition of the septic patient is.

Prognostic markers: Procalcitonin (PCT) and pro-inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 are important clinical prognostic markers in patients with systemic sepsis[11].

There has been strong correlation between serum concentrations of pro-inflammatory mediators and scores of severity of illness[12]. In spite of this, most of these mediators are not established for clinical decision making due to their short half-life[13].

Casey *et al*[14] designed a biologic score for application in septic patients. It included levels of endotoxin, IL-1B, TNF-α and IL-6. It proved a strong correlation with mortality in septic patients. However, the same goal could be achieved by estimation of blood lactate level as an easier and cheaper test.

Nylen *et al*[15] presented the first evidence that PCT, one of the best prognostic markers of sepsis[16], may actually be a sepsis mediator and could have an integral role in the inflammatory process and its prognostic categorization.

It has been shown that *in vitro* and *in vivo* induction of cytokines leads to the rapid release of PCT which has a long half-life[17,18].

Ugarte *et al*[19] showed that PCT concentration on the first day of the diagnosis of sepsis, severe sepsis or septic shock was significantly higher in non-survivors than in survivors. Proving a strong correlation between PCT and survival of septic patients.

Using stepwise discriminant analysis, PCT was proved to be the best single predictor of outcome in patients with systemic sepsis, as it allocated survivors in 95.8% and non-survivors in 83.3% of patients, with an overall prediction accuracy of 80%[20].

There has been recent reports of altered outcome in sepsis due to the release of lipo-polysaccharide binding protein, bacterial permeability inducing protein, and other key proteins which may result in altered disease susceptibility and severity: as heat shock protein 70 and nitric oxide synthase[21].

It has long been appreciated that many patients with sepsis demonstrate defects in coagulation and fibrinolytic systems. These are manifested as anti-thrombin Ⅲ, protein C, and Protein S and the consumption of fibrinogen, together with the appearance of disseminated intravascular coagulation. More recently, there has been a report of a randomized multicenter trial which has examined the use of a novel human activated protein C during the management of patients with severe sepsis[5]. A total of 1690 patients with severe sepsis were enrolled into the study; 850 patients received the protein C preparation and 840 received placebo. The mortality rate was decreased from 30.83% in the control group to 24.71% in the active treatment group, an effect which was statistically significant. This report may clearly have major implication for the prognostic categorization and management of patients with systemic sepsis.

The general interest in genetics culminated in the publication of the findings of the human genome project which appeared in February 2001 issue of *Nature*. The precise structure of 30 000 genes which make up the human DNA bank is now known and can be downloaded from the USA National Human Genome Project Internet Site. Such knowledge will prove useful because it will increase the understanding of the etiology and pathology of many disease processes. Because the technology used to identify the genetic make-up of individual patients is now advancing so rapidly, it will soon be possible to identify more markers in patients and will allow prognostic stratification of septic patients for future trials of new therapeutic approaches[21].

Regional VO_2/DO_2 relationship

The technique of gastric or sigmoid tonometry measures intramucosal pH (Phi) by allowing the equilibration of CO_2 pressures between fluid or air-filled balloon and the interstitial fluid of the mucosa. Measurement of gut intramucosal CO_2 can be also achieved through air introduced directly into the gut (balloonless air tonometry), which equilibrates with the interstitial fluid of the mucosa and is then aspirated from the stomach[22]. Measurement of CO_2 content of fluid aspirated from the stomach has been also described by Mohsenifar *et al*[23]. Both later methods avoid the use of commercial expensive tonometry catheters costing $ 200 each[24].

Phi may decrease due to changes in blood flow to the stomach or sigmoid mucosa due to splanchnic ischemia in shocked patients. Phi appears to be useful for prognostic categorization of ICU patients with systemic sepsis based on serial measurements[25]. The author of the present review has shown that Phi values were significantly lower in septic patients with MODS on admission to the ICU than in patients with no organ dysfunction[10].

Global vs regional VO_2/DO_2 relationship

Assessment of both global and regional VO_2/DO_2 relationships can combine both sides of the coin in prognostic categorization of ICU patients with systemic sepsis. However, there is no gold standard for the detection of tissue hypoxia. There are no specific clinical signs and no clearcut threshold for any single laboratory test. But a multitude of tests combined with sequential clinical evaluations of septic patients may be the best way for their prognostic categorization in the ICU. So, management of patients with severe sepsis or septic shock may be through haemodynamic-oriented or splanchnic-directed therapy added to sequential repeated clinical interpretations. This is a gold standard for both therapy and prognostic categorization of ICU patients with systemic sepsis.

SEVERITY SCORING SYSTEMS IN ICU SEPTIC PATIENTS

Severity scoring systems provide numerical scores that

Table 2 Multiple organ dysfunction score

Organ system	Score				
	0	1	2	3	4
Respiratory PaO_2/FiO_2	> 300	226-300	151-225	76-150	≤ 75
Renal creatinine (μmol/L)	≤ 100	101-200	201-350	251-500	> 500
Hepatic bilirubin (μmol/L)	≤ 20	21-60	61-120	121-240	> 240
Cardiovascular PAR[1]	< 10.0	10.1-15	15.1-20	20.1-30	> 30.0
Cardiovascular HR (beats/min)	< 120	120-140	> 140	Dopamine > 3 mg/g per min	Lactate > 5 mmoL/L
Hematologic platelet count (/L)	> 120	81-120	51-80	21-50	≤ 20
Neurologic Glasgow coma score	15	13-14	10-12	7-9	≤ 6

[1]Pressure-adjusted heart rate (PAR): Product of the heart rate multiplied by the ratio of the right atrial pressure to the mean arterial pressure.

Table 3 Sequential organ failure assessment score

Organ system	Score				
	0	1	2	3	4
Respiratory PaO_2/FiO_2	> 400	≤ 400	≤ 300	≤ 200	≤ 100
Renal creatinine (μmol/L)	≤ 110	110-170	171-299	300-440 urine output ≤ 500 mL/d	> 440 urine output < 200 mL/d
Hepatic bilirubin (μmol/L)	≤ 20	20-32	33-101	102-204	> 240
Cardiovascular hypotension	No hypotension	MAP < 70 mmHg	Dopamine ≤ 5[1] Dobutamine (any dose)	Dopamine > 5[1] or epinephrine ≤ 0.1[1] or norepinephrine ≤ 0.1[1]	Dopamine > 15[1] or epinephrine > 0.1[1] or norepinephrine > 0.1[1]
Hemotologic platelet count (/mL)	> 150	≤ 150	≤ 100	≤ 50	≤ 20
Neurologic Glasgow coma score	15	13-14	10-12	6-9	< 6

[1]Adrenergic agents administered for at least 1 h (doses given are in μg/kg per minute).

describe the impact of patients' illnesses on their physiological reserves.

Most of the severity scoring systems include assessment of major organ system functions. A prolonged period of hypoperfusion of critical organ beds, such as the liver, the brain, the heart, and the gastro-intestinal tract, may give rise to MOD and failure, which is associated with a high rate of morbidity and mortality[26]. It has been shown that the pattern and evolution of organ system dysfunction over the first 3 d of sepsis is significantly related to 30 d mortality.

Two main types of scoring systems have been developed for use in ICU patients: those that focus on describing morbidity as it evolves; organ dysfunction systems, and those that focus on a single end point, survival or mortality[27]. So, severity scoring systems are usually designed to help in the prognostic categorization of critically-ill patients as regards their morbidity or survival.

Morbidity prediction systems

Morbidity prediction systems include a large number of scoring trials by different authors, based on advanced statistical efforts for different populations of critically-ill patients at various centers. We chose to concentrate on three scoring systems that proved useful for clinical applications, namely, the MOD score, the sequential organ failure assessment (SOFA) score, and the logistic organ dysfunction (LOD) score. However, other scoring systems may prove useful and an ideal prediction scoring system has not been reached yet. It should be noted that these systems do not replace serial clinical interpretations of the septic patients.

The MOD score: The MOD scoring system was developed by Marshall *et al*[4] in 1995 (Table 2).

It included six key organ systems and a score of zero to four was given to each organ according to function (zero being normal function and four being the most severe dysfunction), with a maximum score of 24. A mortality rate of 25% was observed for patients with a score of 9-12, 50% for a score of 13-16, 75% for a score of 17-20 and 100% for a score > 20. The detailed analysis of the results of daily scoring demonstrated the prognostic insights gained by adopting this system[28].

A revision[29] of this score has abandoned the cardiovascular parameter (pressure-adjusted heart rate) in favour of a mixed cardiovascular parameter (Table 3) as follows: 0 = heart rate < 120 beat/min; 1 = heart rate > 120 and < 140 beat/min; 2 = heart rate > 140 beat/min; 3 = need for inotrope: (dopamine > 3 μg/kg per minute), and 4 = lactate > 5 mmoL/L. The Revised MOD scoring system proved to be of value, because pressure adjusted heart rate cannot be measured in a significant proportion of ICU patients due to the absence of central venous monitoring. In fact, approximately one half of the patients in the original Marshall *et al*[4] study could not have a cardiovascular component calculated.

It is recommended that the MOD score and its Re-

Table 4 Logistic organ dysfunction score

Organ system	LOD points						
	Increasing severity/decreasing values			Organ dysfunction free		Increasing severity/increasing values	
	5	3	1	0	1	3	5
Neurologic Glasgow coma score	3-5	6-8	9-13	14-15			
Cardiovascular heart rate (min)	< 30 or < 40		40-69	70-89	30-139	⩾ 140 or 240-269	
Systolic blood pressure (mmHg)				90-239		⩾ 270	
Renal							
Serum urea (g/L)				< 6	6-9.9	10-19.9	⩾ 20
Serum urea nitrogen (mmol)				< 6	6-9.9 or 106-140	10-19.9 or ⩾ 141 or ⩾ 10	⩾ 20
Creatinine (μmol)				< 106			
Urine output (l/d)	< 0.5		0.5-0.74	0.75-9.99			
Pulmonary PaO_2/FiO_2 on MV		< 150	⩾ 150	No ventilation, no			
or CPAP PaO_2 (kPa)/FiO_2		(< 19.9)	(⩾ 19.9)	IPAP or no CPAP			
Hematologic							
White blood cell count ($\times 10^9$/L)		< 1.0	1.0-2.4 or < 50	2.5-49.9	⩾ 50.0		
Plateltes ($\times 10^9$/L)				⩾ 50			
Hepatic bilirubin (μmol/L)				< 34.2	⩾ 34.2		
Prothrombin time, seconds above standard (% of standard)			(< 2.5%)	⩽ 3 (⩾ 25%)	> 3		

IPAP: Inspiratory positive airway pressure; CPAP: Continuous positive airway pressure; LOD: Logistic organ dysfunction.

vised form should be measured at the same point in time every day (first morning values). The use of measurements at one particular time avoids capturing momentary physiological changes unrelated to patient condition.

In a small study, Jacobs *et al*[29] compared daily MOD scores to daily Acute Physiology and Chronic Health Evaluation (APACHE) Ⅱ scores in 39 septic-shock patients from one Saudi Arabian ICU. The authors found that the maximum MOD score and the maximum change in the score from admission, both discriminated (the ability to predict mortality in one individual patient) very well between survivors and non survivors, whereas APACHE Ⅱ score did not.

To summarize, the MOD score and its revised form can be used to represent organ dysfunction at baseline and during ICU stay. They can also significantly contribute to the prediction of hospital or ICU mortality.

The SOFA score: The SOFA score (Table 3) was developed in 1994 during a Consensus Conference organized by the European Society of Intensive Care and Emergency Medicine, in an attempt to provide a means of describing the degree of organ failure over time in individuals and groups of ICU septic patients.

It was initially termed the Sepsis-related Organ Failure Assessment score, but it has been realized that it could be applied to non-septic patients as well.

It includes scores for six organ systems where a score of zero is given for normal function and a score of four is given for the most abnormal one. The worst values on each day are recorded and organ function total score can thus be monitored over time[27].

Vincent *et al*[30] in 1998 working on "sepsis-related" problems published the first evaluation of the SOFA score. They found that infected patients had more severe organ dysfunctions compared to those without infection. Antonelli *et al*[31] in 1999 proved that the mean total maximum SOFA score was significantly higher for non-survivors than survivors denoting a high discriminative power (the ability to predict mortality in an individual patient). Because the total maximum SOFA score can be easily calculated daily for the patient, no restriction based on the patients' ICU length of stay is necessary. So, increasing organ dysfunction as measured by the SOFA score consistently correlates with increasing mortality. The SOFA score is also a reliable measure of organ dysfunction at ICU admission.

There were some early published studies that have since examined the utility and accuracy of the SOFA score, which proved that maximum SOFA score and increasing SOFA score are highly prognostic for stratification of critically ill patients including septic patients[32-34].

The LOD score: The LOD score (Table 4) was developed in 1996 using multiple logistic regression applied to selected variables from a large database of ICU patients[35]. The score consists of six organ systems and 12 variables with a maximum of 22 scoring points. If no organ dysfunction is present the score is zero, rising to a maximum of five as the worst severity organ dysfunction.

For maximum dysfunction of the pulmonary and hematologic systems, a maximum of three points can be given for the most severe levels of dysfunction and for the liver, the most severe dysfunction only receives one point. The variables had been recorded as the worst value of each organ dysfunction in the first 24 h of ICU admission. A reference table converts the score to a probability of hospital mortality, the relationship being sigmoid. The

Table 5 Acute physiology score in Acute Physiology and Chronic Health Evaluation Ⅱ

Physiological variable	High abnormal range					Low abnormal range			
	4	3	2	1	0	1	2	3	4
Temperature-rectal (℃)	≥ 41	39-40.9		38.5-38.4	36-38.4	34-35.9	32-33.9	30-31.9	≤ 29.9
Mean arterial pressure (mmHg)	≥ 160	130-159	110-129		70-109		50-69		≤ 49
Heart rate (ventricular response)	≥ 180	140-179	110-139		70-109		50-69	40-54	≤ 39
Respiratory rate (non-ventilated or ventilated)	≥ 50	35-49		25-34	12-24	10-11	6-9		≤ 5
Oxygenation									
A-a DO_2 (mmHg)									
FiO_2 ≥ 0.5	≥ 500	350-499	200-349		< 200				
Record									
A-a DO_2 (mmHg)									
FiO_2 < 0.5 record only PaO_2					PO_2 > 70	PO_2 (6-70)		PO_2 (55-60)	PO_2 < 55
Arterial pH	≥ 7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	< 7.15
Serum sodium (mmol/L)	≥ 180	160-179	155-159	150-154	130-149		120-129	111-119	< 7.15
Serum potassium (mmol/L)	≥ 7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		< 2.5
Serum creatinine (10 mg/L) (double point score for acute renal failure)	≥ 3.5	2-3.4	1.5-1.9		0.6-1.4		< 0.6		
Hematocrit (%)	≥ 60		50-59.9	46-49.9	30-45.9		20-29.9		< 20
White blood count (total/mm^3) (in 1000)	≥ 40		20-39.9	15-19.9	3-14.9		1-2.9		< 1
GCS: score = 15 minus actual GCS									
Total APS: Sum of the 12 individual variable points									
Serum HCO_3 (venous-mmol/L) (not preferred, use if no ABG)	≥ 52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	< 15

GCS: Glasgow coma score; APS: Acute physiology score .

score can thus discriminate between survivors and non-survivors.

The LOD score aims to achieve similar goals to the MOD score, namely, to quantitatively and qualitatively describe organ dysfunction. The goal is to provide a tool that can itself provide a useful outcome measure (e.g., improvement/resolution of organ dysfunction) rather than merely predicting mortality. Though, not originally described as a serial measure, it appears that the LOD score may hold the most promise for patient outcome in the future[21].

Mortality/survival prediction systems

Mortality/survival prediction scoring systems include a large number of scoring trials by different authors, based on advanced statistical efforts including equations for different populations of critically-ill patients. We chose to concentrate on important examples which are useful for clinical prognostic stratification of mortality/survival of patients namely; the APACHE scores, the therapeutic intervention scoring system, the simplified acute physiology score (APS) and the mortality probability models. However, other scoring systems may prove useful and an ideal scoring system for mortality/survival prediction has not been reached yet. It should be noted that these systems do not replace serial clinical interpretations of the septic patients.

The APACHE scoring systems

The APACHE Ⅱ scoring system was developed by Knaus *et al*[36] in 1985 as a refinement of the original APACHE score. It consists of: APS, Age points, and Chronic Health points. The reduced number of physiological variables of APS from 34 in the original APACHE to 12 in APACHE Ⅱ was achieved by a multivariate analysis. The total physiological derangement score is the sum of the individual scores (0-4) for each variable, except the Glasgow coma scale (GCS) where the score is 15 min the GCS. The most deranged value in the first 24 h of ICU admission is used as the scoring for each variable (Table 5). The total physiological derangement score is added to a score of age (0 to 6) and a chronic health score for patients with severe organ insufficiency (2 to 5 dependent upon admission status) as shown in Table 5 and Figure 1. The number of disease groups was 56. The total APACHE Ⅱ score ranges between zero and 71 points. Points of 25 or less denote less than 50% mortality while points of 35 or more denote more than 80% mortality. However, some investigators have used APACHE Ⅱ scoring over time to assess the prognosis of individual patients.

Generally, data of the APACHE Ⅱ score are computed through the following equation to deliver the final risk of hospital mortality:

(R/1-R) = -3.517 + (APACHE Ⅱ × 0.146 + S + D)

where: R = Risk of hospital death, S = Risk imposed by emergency surgery, and D = Risk imposed by specific disease.

Under the APACHE Ⅱ system, the predicted individual death rate is based on a decision criterion of 0.50.

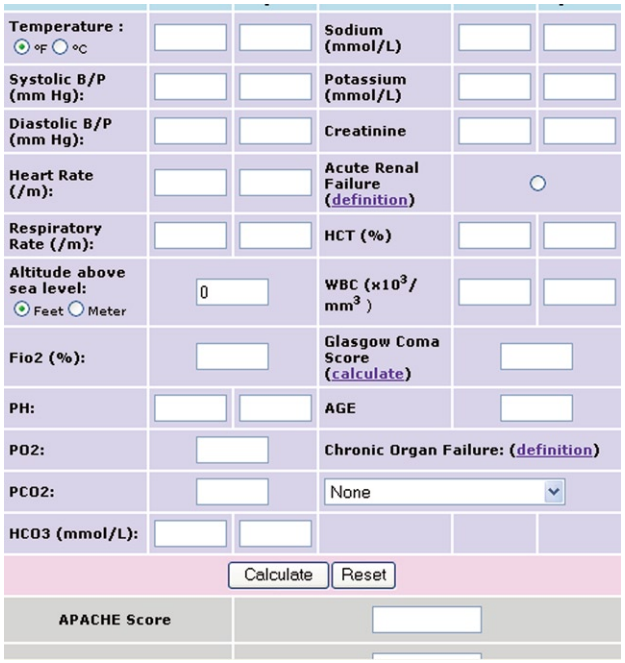


Figure 1 Calculator of Acute Physiology and Chronic Health Evaluation Ⅱ scoring system.

Any patient with an estimated risk of death greater than 0.50 is simply expected to die.

Although the APACHE Ⅱ score provides valuable information about the severity of illness of patient groups, they provide little information about the severity of illness of individual patients[37]. For example, an APACHE Ⅱ score of 20 does not tell whether the patient has severe renal failure or acute respiratory failure, whereas analysis of component scores of an organ dysfunction score as SOFA will provide an accurate description of the patients' disease status. This does not mean that organ dysfunction scores as the SOFA score should replace APACHE Ⅱ score but that the two scores can provide different information and may be used to complement each other[27].

In 1991, Knaus *et al*[38] published a further refinement to their severity of illness scoring system termed APACHE Ⅲ (Table 6). Turning first to the APS, they added some variables and eliminated some parameters. Additional weights were assigned to the extremes of physiological measures. For example, the risk associated with extremely high readings is different from that associated with equally low readings. GCS variables were also refined. The authors also re-weighted age and derived an extended chronic health co-morbidity score.The number of disease groups was increased to 94.The APS in APACHE Ⅲ ranged between zero and 252 points while the total score reached 299 points by adding 24 points for age and 23 points for chronic health evaluation.

The equation of hospital prediction mortality by APACHE Ⅲ differed from that of APACHE Ⅱ and included a risk of location denoting the condition of transference of the patient from a previous locality, as such: R/1-R = (APACHE Ⅲ Score × 0.053) + Risk of emergency Surgery + Risk of specific disease category + Risk of patient location.

Similar to APACHE Ⅱ score, the predicted death rate of the APACHE Ⅲ score is based on a decision criterion of 0.50 with predicted mortality if R exceeds 0.50.

Independent validation of APACHE Ⅲ has been undertaken by a number of studies[39-43], which proved acceptable discrimination performance (the ability to predict mortality in individuals as measured by the area under a receiver operating characteristic curve) and inadequate calibration power (the ability to predict mortality in a large population as measured by a goodness-of-fit test).

A critical prognostic importance of APACHE Ⅲ, may be based on the premise that the changes in APS would reflect the patient response to therapy. The daily APS component of the risk equation would be given by the formula:

Daily risk = day 1 APS + current day APS + change in APS since yesterday

Day 1 APS is a significant predictor of hospital mortality, but its relative influence decreases dramatically over time. The current day APS, as the most important single factor, should be measured retrospectively as scoring values are the most deranged in any 24 h period.

When the daily risk is added to the remaining patient variables included in the APACHE Ⅲ score, the coefficients of each variable were established resulting in equations for d 1-7 of ICU admission. Research is going on to extend the model beyond day 7.

Changes in protocols and practices within ICUs prompted a full review and updating of all the mortality APACHE Ⅲ equations[38] by using the same variables as APACHE Ⅲ with added new variables: mechanical ventilation, thrombolysis, impact of sedation on GCS, together with rescaling of GCS and oxygenation index. Updating used the largest group of patients ever used for APACHE equations modeling care from 104 ICUs in 45 hospitals, with a total of 131 618 observations. The two used statistical techniques were logistic and linear regressions. The result was a new version called APACHE Ⅳ[44], whose calculator is shown in Figure 2.

In addition, there were several changes made for the modeling process used in APACHE Ⅲ. The first involved the laboratory values that were previously considered as "normal". That is, if a measurement was missing, then the value of the previous day was carried forward. If the previous day value was also missing, then the value from 2 d back was carried forward, *etc.* the second change excluded patients transferred from another ICU, because extensive clinical interventions and life support before ICU admission biases the prognostic implications of the first ICU day physiologic measures. The third change was measurement of previous length of hospital stay (LOS) as a continuous rather than an integer value. Previous LOS was defined as the square root of (ICU admission date/time-Hospital admission date/time). Fourth, to more precisely determine the impact of neurological

Table 6 Acute physiology score in Acute Physiology and Chronic Health Evaluation Ⅲ scoring system

Parameter	Value range	Points	Parameter	Value range	Points
Core temperature (℃)	0-32.9	20	Plasma bilirubin (μmol/L)	0-34	0
	33.0-33.4	16		35-51	5
	33.5-33.9	13		52-85	6
	34.0-34.9	8		86-135	8
	35.0-35.9	2		136 plus	16
	36.0-36.9	0			
	40 or more	4			
Heart (r/min)	0-39	8	Urine volume (mL/24 h)	0-399	15
	40-49	5		400-599	3
	50-99	0		600-899	7
	100-109	1		900-1499	5
	110-119	5		1500-1999	4
	120-139	7		2000-3999	0
	140-154	13		4000 plus	1
	155 or more	17			
Mean blood pressure (mmHg)	0-39	23	Plasma Creatinine	0-43	3
	40-59	15	(μmol/L)	44-132	0
	60-69	7	(if no acute renal failure)	133-171	2
	70-79	6	or in ARF	172 or more	7
	80-99	0	(< 410 mL urine vol/24 h)		
	100-119	4			
	120-129	7		0-132	0
	130-139	9		133 or more	10
	140 or more	10			
Respiratory (r/min)	0-5	17	Arterial PO_2 (kPa)	0-6.66	15
(zero points for 6-12/min	6-11	8	(Inspired O_2 < 50%)	6.67-9.32	5
rate if on ventilation)	12-13	7	or alveolar/arterial PO_2	9.33-10.6	2
	14-24	0	difference kPa	10.7 plus	0
	25-34	6	($Pa-PaO_2$)		
	35-39	9	(Inspired O_2 > 50%)	0-13.2	0
	40-49	11		13.3-33.2	7
	50 or more	18		33.3-46.5	9
				46.6-66.6	11
				66.7 and over	14
White cell count (× 10^9/L)	0-0.9	19	Age (yr)	0-44	0
	1.0-2.9	5		45-59	5
	3.0-19.9	0		60-64	11
	20.0-24.9	1		65-69	13
	25 or more	4		70-74	17
				75 or more	24
Haematocrit (%)	0-49.9	0	Chronic health evaluation	Cirrhosis	4
	50 or more	3	(do not score in elective	Immunosuppression	10
			surgery patients)	Leukaemia	10
				Multiple myeloma	10
Plasma sodium (mmol/L)	0-119	3		Metastaic cancer	11
	120-134	2		Lymphoma	13
	135-154	0		Hepatic failure	16
	155 or more	4		AIDS	23
Plasma albumin (g/L)	0-19	11	Neurological score	Use matrix	0-48
	20-44	0			
	45 or more	4			
Acid base status	Use matrix	0-12			

derangement, a variable was added indicating whether a GCS could not be assessed due to sedation. The most important change involved the new categorization of disease groups. Based on the frequency of selected diagnosis and their mortality rate, the existing 94 groups were expanded to 116[3,44]. However, the major changes to the equations included the addition of new variables, the recalling of previous LOS, and increasing the number of disease groups from 94 to 116.

The APACHE systems are the only validated ICU risk adjustment models that provide performance information about 2 separate outcomes of care; mortality and ICU LOS, the APACHE Ⅳ model is the most recent version. Researches are enthusiastic nowad to discontinue the use of APACHE Ⅱ and Ⅲ and move to the more contemporary and accurate APACHE Ⅳ, now that both the score and the two predictions are in public[44].

The Therapeutic Intervention scoring system

The Therapeutic Intervention scoring system was devel-

Figure 2 Calculator of Acute Physiology and Chronic Health Evaluation Ⅳ scoring system.

oped by Cullen *et al*[45] in 1974 as the earliest severity scoring system. It is composed of 76 monitoring and therapeutic parameters. Each modality is assigned a weighted score, ranging from 1 to 4, depending on the intensity of intervention. For example, a peripheral *iv* line or a urinary catheter is assigned one point. A central venous line or two peripheral *iv* catheters are assigned two points. A central *iv* line for hyperalimentation or the application of a chest tube is assigned three points. A pulmonary artery catheter for vaso-active drug infusion is assigned four points. Each modality is assigned to one of three categories: active therapy, ICU monitoring or standard floor care. Points are totaled and TISS score is obtained by a calculator (Figure 3). Patients can be then stratified into one of four classes based on the number of TISS points. TISS is based on the premise that, regardless of the diagnosis, the amount of therapy based on the amount of monitoring reflects the degree of physiological impairment. The TISS does not predict outcome on patient admission to the ICU. However, trends of the score over the first three d in ICU correlate well with survival. If the TISS points do not improve at the third day, the likelihood of death increases. So, it discriminates between survivors in whom the score falls progressively and non-survivors in whom the score remains static. Moreover, the TISS can identify those patients who require monitoring only.

The TISS is used most frequently in conjunction with the APACHE systems. So, Both together can be used to evaluate concordance between severity of illness and quantity of needed therapy. Either the TISS alone or in conjunction with the APACHE scoring systems can be used for prognostic categorization of patients with systemic sepsis.

Simplified APS

In 1984, Le Gall *et al*[46], published the Simplified Acute Physiology Score. It was designed to overcome some of the problems of APS of the APACHE systems. The authors selected the 13 "most easily measured" physiological variables available in 90% of patients from a previous survey employing the APS that they had conducted. SAPS scores these variables (0-4) in an identical manner to the APS of the APACHE Ⅱ system, adds a score for age (0-4) and replaces respiratory rate or the P(A-a) O_2 which is difficult to measure with a fixed score of 3 for patients receiving mechanical ventilation or CPAP. The most abnormal values from the first 24 h of ICU admission are taken as the total scoring value. Le Gall *et al*[46] concluded that SAPS performed at least as well if not better than APS of the APACHE system but was more useful as it was much simpler. They stressed that SAPS is applicable to a wide range of pathologies but that its

(Therapeutic Intervention Scoring System - Update 1983)

4 points		3 points	
a. Cardiac arrest and/or countershock within past 48 h	○ yes ○ no	a. Central iv hyperalimentation (includes renal, cardiac, hepatic failure fluid)	○ yes ○ no
b. Controlled ventilation with or without PEEP	○ yes ○ no	b. Pacemaker on standby	○ yes ○ no
c. Controlled ventilation with intermittent or continuous muscle relaxants	○ yes ○ no	c. Chest tubes	○ yes ○ no
d. Balloon tamponade of varices	○ yes ○ no	d. IMV or assisted ventilation	○ yes ○ no
e. Continuous arterial infusion	○ yes ○ no	e. CPAP	○ yes ○ no
f. Pulmonary artery catheter	○ yes ○ no	f. Concentrated K^+ infusion via central catheter	○ yes ○ no
g. Atrial and/or ventricular pacing	○ yes ○ no	g. Nasotracheal or orotracheal intubation	○ yes ○ no
h. Hemodialysis in unstable patient	○ yes ○ no	h. Blind intratracheal suctioning	○ yes ○ no
i. Peritoneal dialysis	○ yes ○ no	i. Complex metabolic balance (frequent intake and output)	○ yes ○ no
j. Induced hypothermia	○ yes ○ no	j. Multiple ABG, bleeding, and/or STAT studies (> 4 shift)	○ yes ○ no
k. Pressure-activated blood infusion	○ yes ○ no	k. Frequent infusion of blood products (>5 units /24 h)	○ yes ○ no
l. G-suit.	○ yes ○ no	l. Bolus iv medication (nonscheduled)	○ yes ○ no
m. Intracranial pressure monitoring	○ yes ○ no	m. Vasoactive drug infusion (1 drug)	○ yes ○ no
n. Platelet transfusion	○ yes ○ no	n. Continuous antiarrythmia infusions	○ yes ○ no
o. IABP (Intra Aortic Balloon Pressure)	○ yes ○ no	o. Cardioversion for arrythmia (not defibrillation).	○ yes ○ no
p. Emergency operative procedures (within past 24 h)	○ yes ○ no	p. Hypothermia blanket	○ yes ○ no
q. Lavage of acute GI bleeding	○ yes ○ no	q. Arterial line	○ yes ○ no
r. Emergency endoscopy or bronchoscopy	○ yes ○ no	r. Acute digitalization - within 48 h	○ yes ○ no
s. Vasoactive drug infusion (> 1 drug)	○ yes ○ no	s. Measurement of cardiac output by any method	○ yes ○ no
		t. Active diuresis for fluid overload or cerebral edema	○ yes ○ no

Figure 3 Calculator of Therapeuti Intervension scoring system.

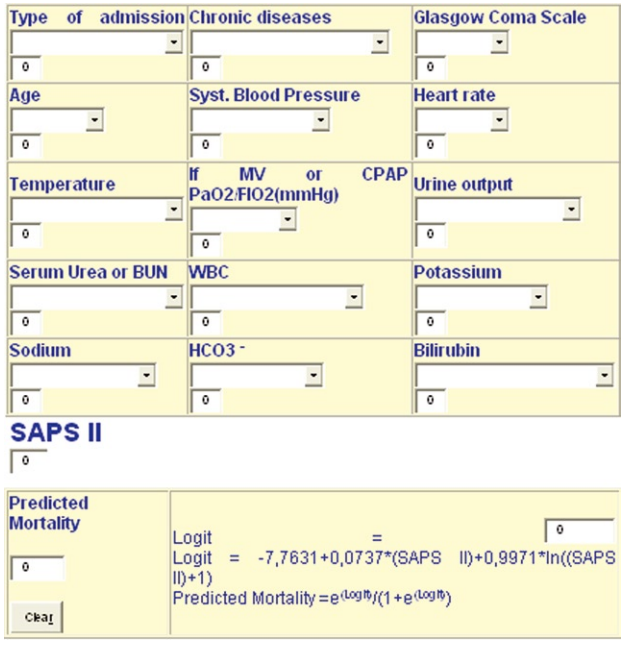


Figure 4 Calculator of Simplified Acute Physiology scoring system Ⅰ.

predictive value and performance can only be applied to groups of patients, not to individual patients.

In 1993, Le Gall *et al*[47] published a refined version of their original SAPS termed SAPS Ⅱ whose calculator is shown in Figure 4, and the variables were 17 (12 physiological, age, type of admission and 3 chronic health diagnosis).

The main advantage of SAPS Ⅱ over APACHE Ⅲ is the ability to accurately predict mortality in stratified groups of patients without recourse to defining a single diagnosis, which is only possible in a minority of patients.

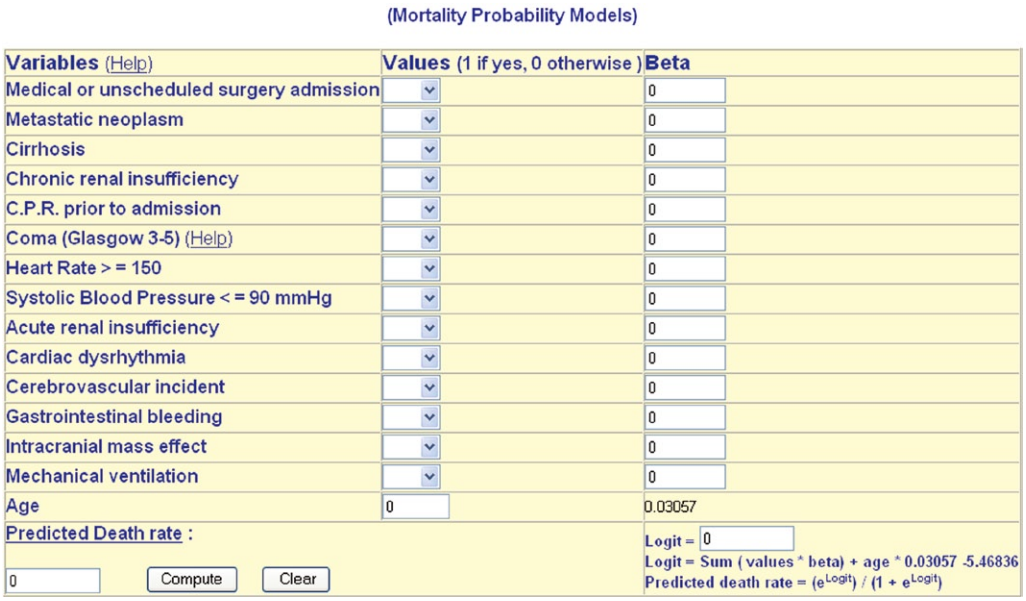
It is clear that SAPS Ⅱ can be useful for prognostic stratification for groups of critically ill patients including those with systemic sepsis. It can also be useful for guiding therapy, comparing the management of these patients overtime and comparing ICU performance of groups of patients in different ICU's.

The SAPS Ⅱ score varies between zero and 163 points: 116 points for physiological variables, 17 points for age and 30 points for previous diagnosis.

SAPS Ⅲ assesses 12 physiological variables: at the first 24 h of ICU admission as SAPS Ⅱ, and includes weighing for pre-admission health status and age. It has been poorly studied, with the exception of some formal analysis of data accuracy in the original publication and external validation studies[48,49].

The mortality probability models

In 1985, Lemeshow *et al*[50] published their first attempt at an outcome prediction model. They actually developed four models: MPM_0 (probability of death from data collected at ICU admission), MPM_{24} (probability of death from data collected at 24 h), MPM_{48} (probability of death from data collected at 48 h) and MPM_{0T} (probability of death "overtime" based on MPM_0 and the change in probability between MPM_0 and MPM_{24}, and between MPM_{24} and MPM_{48}). Patients whose probability of mortality started high and remained high, or increased by > 10% had a very high actual mortality. It deserves mentioning that for ICU triage purposes, MPM_0 is the most valid model at present.



(Mortality Probability Models)

Variables (Help)	Values (1 if yes, 0 otherwise)	Beta
Medical or unscheduled surgery admission		0
Metastatic neoplasm		0
Cirrhosis		0
Chronic renal insufficiency		0
C.P.R. prior to admission		0
Coma (Glasgow 3-5) (Help)		0
Heart Rate > = 150		0
Systolic Blood Pressure < = 90 mmHg		0
Acute renal insufficiency		0
Cardiac dysrhythmia		0
Cerebrovascular incident		0
Gastrointestinal bleeding		0
Intracranial mass effect		0
Mechanical ventilation		0
Age	0	0.03057
Predicted Death rate : 0 Compute Clear		Logit = 0 Logit = Sum (values * beta) + age * 0.03057 -5.46836 Predicted death rate = $(e^{Logit}) / (1 + e^{Logit})$

Figure 5 Calculator of mortality probability model.

In common with APACHE and SAPS systems, MPM had low sensitivity (ability to predict those patients who are going to die) but high specificity (ability to predict those patients who are going to live).

Lemeshow *et al*[51] published a revision of their MPM termed MPM Ⅱ. They employed a near identical method to that they had used in developing their original MPM. The authors initially developed MPM $Ⅱ_0$ and MPM $Ⅱ_{24}$, deciding to temporarily abandon the MPM_{48} and MPM $_{0T}$ of the original model.MPM $Ⅱ_0$ was determined by 15 variables. Lemshow *et al*[52] in 1994, found that patients alive but still requiring to be on ICU at 24 h differed markedly from those who had either died or been discharged. They emphasized that MPM $Ⅱ_{24}$, including 13 variables, is a companion model to MPM $Ⅱ_0$ and represents a different population of patients. The authors argue that this approach exposes one of the main weaknesses of the APACHE and SAPS models, which take the worst data from the first 24 h of ICU admission, and failed to differentiate between the two originally observed populations.

The following year, Lemeshow *et al*[53] published two further models based upon their data set, MPM $Ⅱ_{48}$ and MPM $Ⅱ_{72}$. Both these models use the same 13 variables as MPM $Ⅱ_{24}$. They pointed out that the probability of death changes with time, while an APACHE or SAPS score is only valid at 24 h of ICU admission. They also emphasized that an ICU patient whose condition failed to improve day after day, was in fact deteriorating and had an increasing risk of death. This well recognized clinical phenomenon is accurately modeled over the first 72 h of their ICU stay by MPM Ⅱ. The same could not be said for sequential APACHE Ⅱ scoring. The authors described an on-going process to develop MPM Ⅱ models for successive time points beyond 72 h. (MPM Ⅱ $_{0T}$). Figure 5 shows the calculator of the MPM.

Limitations of scoring systems

Data-base still continues about the accuracy of scoring systems, their efficiency in assessing the severity of illness, and whether they have a prognostic role in the estimation of illness outcome. Additionally, these tools have to be validated in the population in question before they are adopted for outcome prediction and decision-making[53].

The most important potential limitation of scoring systems is the inappropriate interpretation of the score. Clinicians must be aware that the probability of in-hospital mortality based on a particular score relates to a similar group of patients and not to an individual patient. This is important to understand before attempting to use scoring systems in clinical practice. So, although it can be useful to know the predicted mortality of a group of patients with a similar score, we cannot be sure which patients will die and which will survive. A well calibrated model, applied to an individual patient, may for example predict a hospital mortality of 46% for this patient, which just means that for a group of 100 patients with a similar severity of illness, 46 patients are predicted to die, but it makes no statement if the individual patient is included in the 46% who will die or in the 54% that will survive. Consequently, scoring systems should not be used to make predictions for individual cases. Conversely, scoring systems can appropriately be used to assist the clinical decision making as they do allow an objective assessment of a patient's severity of illness, and therefore reflect the likelihood of mortality in a similar cohort of patients. Overall, they should be considered as a fact to assist the clinician.

OVERVIEW

It is now about 30 years since the original APACHE

study was published. Tens of thousands of patients have been studied and mortality prediction models and prognostic categorization morbidity models developed on universal ICU critically ill patients.

Severity scoring systems are usually designed to predict morbidity or mortality in critically- ill patients. Examples of general scoring systems are APACHE Ⅲ; SAPS Ⅱ and MPM Ⅱ. Examples of organ dysfunction scoring systems are MODS, SOFA and LODS. Examples of specific severity scoring systems include Acute Pancreatitis and Acute Lung Injury scores. Biological scores include measurements of serum lactate and PHi. Examples of overtime or dynamic severity scoring systems are APACHE Ⅲ, $MPM_{24\text{-}72}$ and intermediate TISS.

Because general severity scoring systems are developed and validated using admission data from large ICU populations, they are most fitted to predict mortality for groups of ICU patients rather than predicting mortality for individual patients. They are used for determining ICU proficiency (in quality assurance) and treatment efficacy (in clinical practice). Decisions regarding ICU triage are often more dependent on values than probabilities and so, these systems should not determine the utility or futility of ICU for individuals.

Even if a severity scoring index could perfectly predict the mortality of a septic patient from admission data, one should be cautious, because death cannot actually be predicted except just before its occurrence and by that time, there would be little to be gained. By contrast, early prediction of death might be more useful to design patient management. It would be likely to be associated with a greater risk of a false positive result.

Outcome estimates may influence the clinical management. The clinical awareness of the treating physician of a poor outcome for his/her patient may tempt him/her to give less than optimal therapy or to prevent ventilating him or even to withdraw active therapy. To date, however, it is almost impossible to find documented evidence of change in medical practice that have resulted from application of different prognostic scoring systems[21]. There is clearly no "best" severity scoring model, and the performance of such models varies both with time and with the population under study, and so should be periodically addressed. For this, severity scoring systems should be used in conjunction with sequential patient clinical interpretation and clinical assessment of tissue hypoxia for prognostic categorization of critically-ill patients in general and septic patients in particular.

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GUIDELINES FOR BASIC RESEARCH

General anesthesia mediated by effects on ion channels

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Abstract

Although it has been more than 165 years since the first introduction of modern anesthesia to the clinic, there is surprisingly little understanding about the exact mechanisms by which general anesthetics induce unconsciousness. As a result, we do not know how general anesthetics produce anesthesia at different levels. The main handicap to understanding the mechanisms of general anesthesia is the diversity of chemically unrelated compounds including diethyl ether and halogenated hydrocarbons, gases nitrous oxide, ketamine, propofol, benzodiazepines and etomidate, as well as alcohols and barbiturates. Does this imply that general anesthesia is caused by many different mechanisms? Until now, many receptors, molecular targets and neuronal transmission pathways have been shown to contribute to mechanisms of general anesthesia. Among these molecular targets, ion channels are the most likely candidates for general anesthesia, in particular γ-aminobutyric acid type A, potassium and sodium channels, as well as ion channels mediated by various neuronal transmitters like acetylcholine, amino acids amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid or N-methyl-D-aspartate. In addition, recent studies have demonstrated the involvement in general anesthesia of other ion channels with distinct gating properties such as hyperpolarization-activated, cyclic- nucleotide-gated channels. The main aim of the present review is to summarize some aspects of current knowledge of the effects of general anesthetics on various ion channels.

Key words: General anesthesia; Ion channels; γ-aminobutyric acid type A receptors; Hyperpolarization activated cyclic nucleotide; Potassium channels; Glutamatergic ion channels; Sodium channels

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INTRODUCTION

The start of modern anesthesia, through the use of inhaled volatile anesthetics 150 years ago, dramatically revolutionized modern medicine. Dentist Dr. Horace Wells used nitrous oxide for a public demonstration of its powers of intoxication. Another dentist, William Morton, took up Wells' idea of a gaseous anesthetic, together with the suggestion from Charles Jackson to use ether, to perform a widely known public demonstration of ether anesthesia on 16 October, 1846.

The structural diversity of general anesthetics, from simple chemically inert gases to complex barbiturates, has baffled anesthesiologists, and ideas about how these anesthetics might work have been correspondingly confused. In the early stages, the notion that anesthetics worked "nonspecifically" by dissolving in the lipid bi-layer portions dominated. Although this simple idea could explain the structural diversity of general anesthetics, it is now

generally accepted that anesthetics act by binding directly to sensitive target proteins.

Until now, many receptors, molecular targets and neuronal transmission pathways have been shown to contribute to general anesthesia. Among these molecular targets, ion channels are the predominant candidates for general anesthetic effect, in particular γ-aminobutyric acid type A (GABAA), potassium and sodium channels and ion channels activated by acetylcholine, amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid or N-methyl-D-aspartate. In addition, some other ion channels such as hyperpolarization activated cyclic nucleotide (HCN) channels are also involved in general anesthesia (Table 1).

The main aim of the present review is to summarize some aspects of current knowledge about the function of general anesthetics at different ion channels.

GABAA RECEPTORS AND GENERAL ANESTHESIA

Structure and function of the GABAA receptor

The GABAA receptor is composed of five different subunits (α1-6, β1-3, γ1-3, δ, ε, φ, π and ρ1-3) which are encoded by at least 19 mammalian genes, with additional diversity arising in certain regions[1]. In most GABAA receptors, the most common combination of subunits is α, β, and γ, with a ratio of 2:2:1 although the γ subunit may be replaced by δ or ε subunits, particularly in brain regions, as shown in Figure 1. These GABAA receptor subunits are densely packed in the cortex, and receptors with the γ2 subunit comprise more than 40% of all GABAA receptors in the brain[2].

The GABA system is the main inhibitory neurotransmitter pathway in the CNS of mammalian brain, and one-third of all synapses are GABAergic[3]. The GABA system induces inhibition of the central nervous system by generating fast, transient inhibitory postsynaptic currents. Activation of GABAA receptors decreases excitability of the neurons by an influx of chloride, hyperpolarization of the membrane, and shunting of excitatory input. This synaptic inhibition of the GABA system maintains neuronal communication and induces precise timing of action potentials and synchronization of neuronal populations[4,5]. For many years, enhancement of fast inhibition at synapses was widely regarded as the dominate mechanism underlying the effects of many GABAergic drugs.

The α1 and β2 subunit-containing GABAA receptors in the cortex are thought to contribute to the sedative actions of several inhaled anesthetics. Some studies of tool drugs indicate the important role of GABA receptors and its subunits in the anesthetic effect. Tonic current in the thalamic VB neurons may contribute to the sedative action of 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP). Although THIP is not commonly used as an anesthetic, it promotes slow wave sleep and produces analgesic, sedative, hypnotic actions and ataxic properties[6]. GABAA receptors that contain the α4 and δ subunit

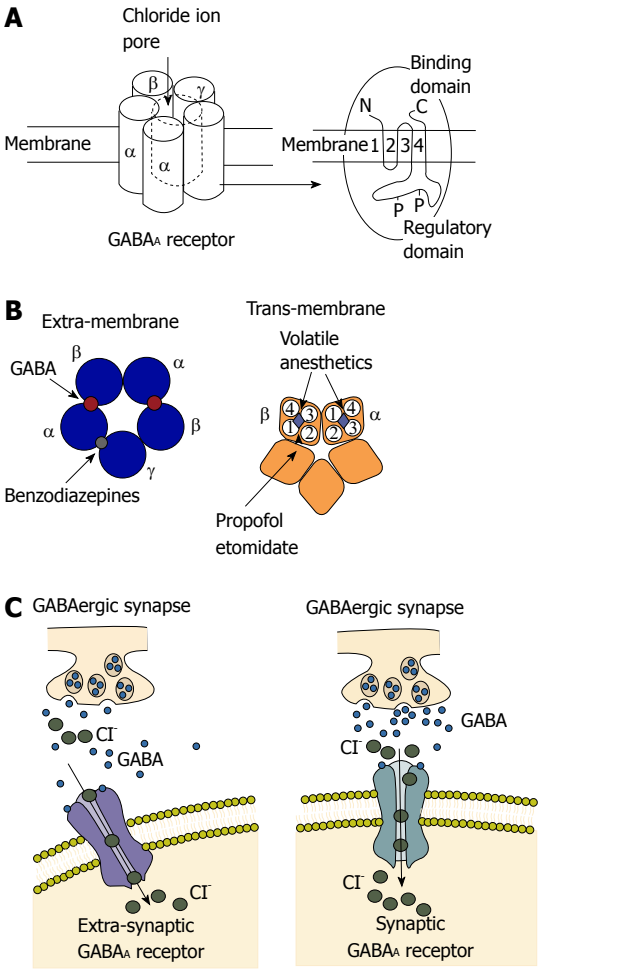

Figure 1 Structure and function of γ- aminobutyric acid type A receptor. A: γ- aminobutyric acid type A (GABAA) receptors commonly contain two α subunits, two β subunits and one γ subunit. Chloride influx through the pore could hyperpolarize the postsynaptic membrane; B: Left: Extra-membrane region of GABAA receptor. The binding sites for GABA are located between α and β subunits and the binding site for benzodiazepines is located between γ and α subunits; Right: Trans-membrane region of GABAA receptor. Four trans-membrane segments form the α subunit. It has been shown that the trans-membrane segment of β subunit is the binding site for propofol and etomidate. This binding site is close to a binding site for volatile anesthetics; C: Activation of the GABAA receptor could increase conductance of the postsynaptic membrane and alter the potential of the membrane because of influx of chloridion. Synaptic receptors could detect GABA at mmol concentration to produce fast inhibitory postsynaptic potentials (IPSPs), and extra-synaptic receptors that detect GABA at μmol concentrations to produce slower IPSPs.

appear to contribute to the sedation effects of THIP. At low concentrations, THIP strongly potentiates the activity of GABAA receptors containing the δ subunit, and enhances a tonic conductance generated by α4δ GABAA receptors[7]. Rotarod performance and spontaneous locomotor activity were unimpaired by THIP in α4 subunit knock-out mice[8], which suggests that α4δ subunit containing GABAA receptors are necessary for the sedative and ataxic effects of THIP. THIP enhanced the tonic but not the phasic GABAA receptor currents in VB neurons, and had no effect on nRT neurons[9]. Since the sedative actions of THIP were absent in α4 knock-out mice, it is likely that the tonic current mediated by α4β2δ GABAA

receptors in VB neurons contributes to anesthetic sedation.

Actions of general anesthetics on GABAA receptors

The enhancement of GABA-activated chloride currents is the main effect of some intravenous general anesthetic such as propofol and etomidate, decreasing neuronal activity by producing hyperpolarization of the neuronal membrane. This is in agreement with the finding that etomidate-mediated sedation also depends on GABAA receptors containing the β2 subunit[9,10], although the specific contribution of thalamic β2 subunits to this effect is uncertain. Propofol and etomidate also enhance function of GABAA receptors to produce immobility[11-13]. In contrast, gaseous general anesthetics such as xenon, nitrous oxide, cyclopropane as well as ketamine have minimal or no effect on GABAA receptor subtypes[14-18].

Compared to other general anesthetics, volatile anesthetics show low potency to a variety of receptors at clinical concentrations[19]. As a result, the determination of the specific sites of effect of volatile anesthetics is a challenge. In addition, behavioral evaluation with volatile anesthetics has some obvious practical difficulties. Even with these handicaps, it has been demonstrated by some carefully designed studies that isoflurane anesthesia is mediated by GABAA receptors. Volatile anesthetics at clinical concentrations could activate GABAA receptors both *in vitro* and *in vivo*, using heterologous expression systems and the postsynaptic membrane, respectively[20,21]. The depressive effects of isoflurane, enflurane and halothane on rat neocortical neuron activity were studied using *in vivo* recordings of spontaneous action-potential firing and *in vitro* recordings from isolated cortical networks. Sedative concentrations of isoflurane, enflurane and halothane similarly reduced the firing of spontaneous action potentials *in vivo* and *in vitro* by approximately 50%. This reduction in neuronal firing strongly correlated with an increase in GABAergic synaptic inhibition. Anesthetics prolonged the time course of GABAA receptor-mediated spontaneous IPSCs from pyramidal neurons in organ cortical cultures with no effect on their frequency or peak amplitude.

At the spinal level the role of inhibitory GABAA receptors on anesthetics actions has been extensively studied. With the evaluation of motor response, MAC of volatile anesthetics was more significantly affected by spinal injections of glycine receptor antagonists than GABAA receptor antagonists[22].

For many years, the binding site of GABAA receptor for volatile anesthetics is still unclear. The binding site for volatile anesthetics on the GABAA receptor was determined to be a binding pocket for volatile anesthetics, by complementary site directed mutagenesis, using general anesthetics of varying molecular size[23]. With the finding of a binding pocket for general anesthetics, the long-held assumption that general anesthetics worked by a nonspecific mechanism was overturned. Dramatic progress has been made in dissecting the behavioral effects of general anesthetics, in particular the subunit combination of GABAA receptors, on anesthetic effect. GABAA receptors containing the α1β2γ2 subunits are enriched at synaptic sites throughout the brain[24]. This suggests that the enhancement of synaptic activity within the cortex could be responsible for anesthetic sedation. The contribution of the cortex to the sedative properties of inhaled anesthetics was studied by Hentschke and colleagues[25]. In recent studies, an anesthetic binding cavity for volatile anesthetics has been identified, critically involving in the α1 subunit[26,27]. Rudolph *et al*[28], reported that animal behavioral patterns induced by benzodiazepine were moderated by a point mutation on the mouse α1 GABAA subunit. At the same time, barbiturates directly activate and inhibit GABAA receptors by means of positive allosteric modulation depending on their concentration at the receptor. In addition, a mutation in the GABAA α subunit was identified that abolishes the action of barbiturates, although, the potentiating by etomidate on GABAA receptors was not affected. Furthermore, enhancement of GABAA mediated transmissions was also affected by alcohol, indicating an important role of alcohol in mediating its intoxicating effects[29].

The biophysical profile of GABA receptors and their sensitivity to general anesthetics can be dramatically altered by subunit composition[30]. Using chimerical channel construction, Mihic and colleagues discovered a domain, relevant for mediating the effect of volatile anesthetics and etomidate[29], but not propofol[27]. Two key amino acids in GABAA receptor subunits were found to be involved in their interaction with volatile anesthetics. These amino residues may contribute to the molecular binding pockets for general anesthetics[31]. According to important studies, two amino acids in the α1 subunit are the most critical points for general anesthetic effect[27]. Serine 270 is in the trans-membrane segment and while Alanine 291 is near the extracellular regions. For GABAA receptors, replacing Ser 270 with larger amino acid residues in the α1 subunit resulted in a decrease of sensitivity to volatile anesthetics[26,31], while replacement with smaller residues resulted in the opposite effect[26]. Also, replacing the α1 Ser270 residue with histidine resulted in recombinant heteromeric GABAA receptors that were insensitive to isoflurane[26]. However, an additional change to the GABAA receptors, introduced by the α1 (Ser270His) mutation, complicated the interpretation of receptor pharmacology[32]. This problem was addressed by introducing an additional mutation into the α1 subunit, whereby the leucine residue at position 277 was replaced with alanine. This double knock-in mutation, α1 (Ser270His, Leu277Ala), restored normal sensitivity to GABA[29]. These mutations laid the foundation for generating knock-in mice that were partially insensitive to isoflurane. Mice with a double knock-in mutation were used to explain the interaction between GABAA receptors containing α1 subunits to isoflurane anesthesia[33]. Some studies demonstrated that double-mutant mice expressing the α1 (Ser270His, Leu277Ala) subunit was less sensitive

to isoflurane, compared to wild-type controls, indicating the important role of α1 subunit in the hypnotic effect of isoflurane. Interestingly, according to the tail clamp test, the immobilizing effect of isoflurane was not affected in these double-mutant mice. Using cued and contextual fear conditioning, the amnesic effect of isoflurane was also unaffected in the α1 (Ser270His, Leu277Ala) mice, comparing to wild-type control, indicating that this subunit is not critical for amnesia induced by isoflurane. This last finding is in contrast to previous work using mouse mutants in which the α1 subunit was knocked out either globally or in the forebrain alone[34]. In other studies with the α1 subunit knock-out mice, the amnesic effect induced by isoflurane was impaired, indicating the role of α1 subunit in isoflurane amnesia. At the same time, the β subunit of GABAA receptors is also important to the binding site of volatile anesthetics, as well as for the behavioral effects of volatile anesthetics[27,35]. In addition, on the β3 subunit when the asparagine residue at position 265 was replaced with methionine or the methionine at position 286 with tryptophan, GABA current potentiated by enflurane was reduced[35]. With β3 (Asn-265Met) knock-in mice, isoflurane is slightly less effect at inhibiting the righting reflex in β3 (Asn265Met) mice, suggesting the role of the β3 subunit in isoflurane hypnosis. The immobility induced by isoflurane, however, is significantly impaired in these knock-in mice, as measured by hind limb or tail clamp withdrawal reflex. Additionally, in β3 (Asn265Met) mice, heart rate and core temperature were decreased less by isoflurane[36], indicating the role of the β3 subunit in the effect of isoflurane on circulation. Therefore, neuronal depressive effect and cardiovascular effects induced by volatile anesthetics might be mediated by distinct GABAA receptor subunits[37].

Knock-in mutant mice have been used to determine the GABAA subunits responsible for the sedative and hypnotic actions of etomidate. Some studies indicate that amnesic effect induced by etomidate might contribute to the α5 GABAA receptors in hippocampal region, while the sedative effect of etomidate might be due to other GABAA receptor isoforms. GABAA receptors with some structural modifications (the asparagine at position 265 in the β2 or β3 subunits was replaced with serine or methionine, respectively) were insensitive to etomidate *in vitro*[9,10]. Etomidate showed low efficacy in reducing spontaneous loco-motor activity in β2 (Asn265Ser) knock-in mice, indicating that GABAA receptors with the β2 subunit were important for the sedative effect of etomidate[9].

In other studies, sedative property of diazepam has been demonstrated to be mediated by the α1 subunit of GABAA receptors. With some different features from general anesthetics, diazepam produces a sedative effect. GABAA receptors which contained a histidine to arginine mutation at position 101 of the α1 subunit were insensitive to diazepam *in vitro*[28]. Behavioral tests indicated that the sedative effect induced by diazepam were eliminated in knock-in mice that expressed the α1 (His101Arg) mutation[28].

Some other types of GABAA receptors, such as the extra junction GABAA receptors, could be activated by GABA at very low concentrations. Junction GABAA receptors are widely expressed in important brain regions including the hippocampus, thalamus, cortex and cerebellum. Currents mediated by these junctions GABAA receptors are affected by volatile anesthetics at low concentrations[38].

Neuroprotection of anesthetics involve in action of GABA receptors

Recent studies have shown that general anesthetics could produce significant neural protection and/or induce a preconditioning effect against ischemia/reperfusion induced injury. Propofol, a potent antioxidant, has been reported to have neural protective effects, reducing cerebral blood flow and intracranial pressure. Many studies have indicated that propofol pretreatment significantly improves post-resuscitation recovery of neuronal functions. Recent studies have suggested that during the process of resuscitation, the effect of GABAA changes from inhibitory to excitatory, through a mechanism that is closely associated with activation of microglia and down regulation of the K^+-Cl^- transporter. It has been demonstrated that propofol might protect the neurons by inhibiting the transition of GABAergic inhibition into excitation during resuscitation.

POTASSIUM CHANNELS AND GENERAL ANESTHESIA

Structure and function of potassium channels

Mammalian K^+ channel subunits contain two, four or six/seven transmembrane segments, as shown in Figure 2. Members of the two and six/seven transmembrane segments classes are characterized by the presence of a single pore-forming (P) domain, whereas the more recently discovered four transmembrane segment subunits contain two P domains that are arranged in tandem[39-43]. Background K^+ channels are transmembrane K^+-selective ionic pores that are constitutively open at rest and are central to neural function. Background K^+ channels and their regulation by membrane-receptor-coupled second messengers, as well as pharmacological agents, are therefore important in tuning neuronal resting membrane potential, action potential duration, membrane input resistance and, consequently, regulating transmitter release[44,45].

Background K^+ channels are composed of K_{2P} channel subunits, previously called KCNKx subunits, or tandemly arranged P domains in weak inwardly rectifying K^+ channel (TWIK) subunits. Two-pore-domain K^+ (K_{2P}) channel subunits are made up of four transmembrane segments and two pore-forming domains that are arranged in tandem and function as either homo-or heterodimeric channels. This structural motif is associated with unusual gating properties, including background channel activity and sensitivity to membrane stretch. In one-pore-domain K^+ (K_{1P}) channels, four matching P

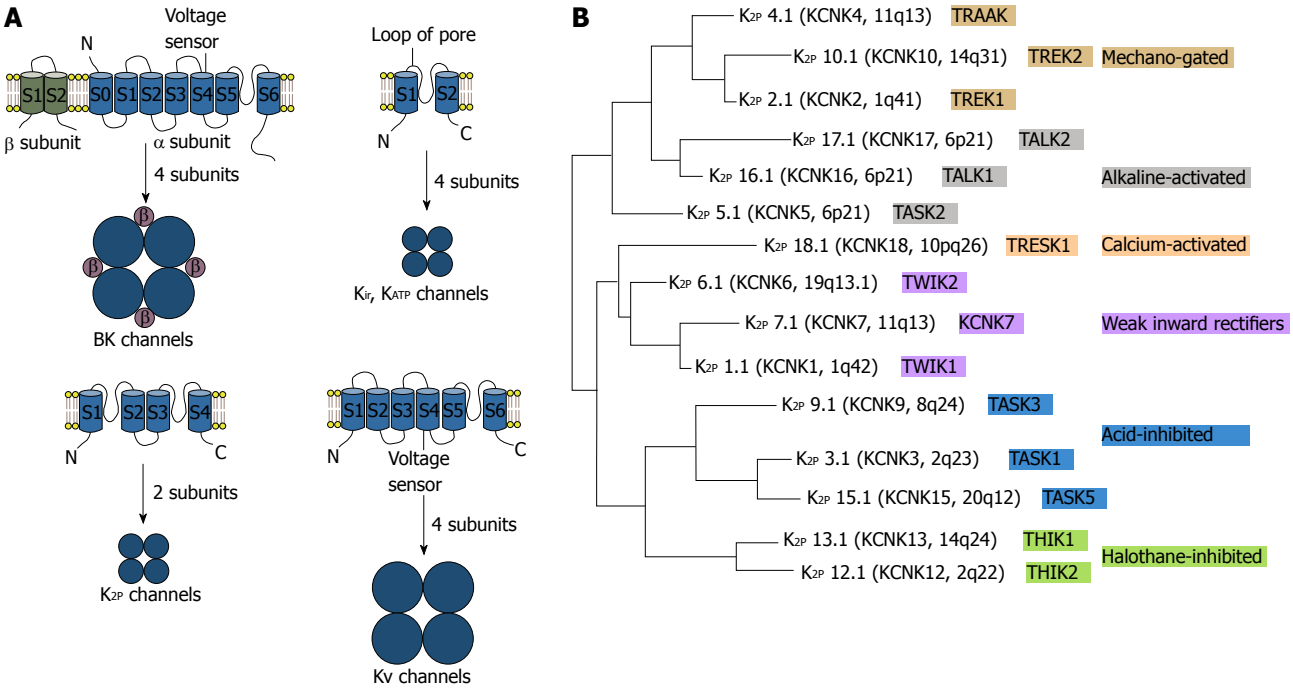

Figure 2 The trans-membrane structures and subunit formulation of the potassium channels and phylogenetic tree of K_{2P} channels in humans. A: The trans-membrane structures and subunit formulation of the potassium channels. BK channels (background) are made up of four α-subunits and the four β subunits. Structures of K_{ir} or K_{ATP} channels are the simplest. Their subunit has two trans-membrane segments connected by a pore loop. Four subunits form a functional channel pore. K_{2P} channels are made of a tetrameric pore made up of two subunits. Subunits of K_V channels have six trans-membrane regions and trans-membrane domain S4 acts as the voltage sensor; B: Phylogenetic tree of K_{2P} channels from humans. The chromosomal localization, nomenclature and functional properties of each subunit are indicated. Different colors indicate the functional subgroups. TASK1: TWIK-related acid-sensitive K^+; K_{2P}: Two-pore-domain K^+.

loops are assembled in homo- or heterotetramers (all subunits have a similar P domain sequence, which contains the residues GYG or GFG), whereas in the dimeric K_{2P} channels, the first pore (P1) and P2 domains have different sequences as exemplified by TWIK1 or TWIK-related acid-sensitive K^+ 1 (TASK1)[46]. Many K_{2P} channels have a phenylalanine or a leucine in the GXG motif (where X represents any amino acid) of the selectivity filter in the P2 domain instead of a tyrosine[40-41,46]. Therefore, in K_{2P} channels, the pore is predicted to have a two-fold symmetry rather than the classical four-fold arrangement of other K^+ channels. Although the selectivity of K_{2P} channels for K^+ over Na^+ is high [permeability ratio (P_{Na}/P_K) < 0.03], these structural differences suggest a more varied permeation and gating compared with K_{1P} channels[39]. K_{2P} channels, including TASK1 and TWIK-related K^+ 1 (TREK1), present an instantaneous current component and a second time-dependent component in response to depolarization[47,48]. Furthermore, TREK1 shows a strong outward rectification in a symmetrical K^+ gradient instead of the linear current to voltage relationship predicted by the GHK equation[49,50]. The outward rectification of TREK1 is attributed to an external Mg^{2+} block, which is present at negative membrane potentials, and to an intrinsic voltage- dependent mechanism[49,50]. Transfection of TREK1 (either splice variant) in HEK cells surprisingly produces two populations of channels with different single-channel conductance (about 40 pS and 100 pS in a symmetrical K^+ gradient)[51]. Therefore, K_{2P} channels diverge from the constant-field GHK current formulation and are characterized by complex permeation and gating mechanisms[49,52].

Recent *in vivo* studies have demonstrated that TREK1, the most thoroughly studied K_{2P} channel, has a key role in the cellular mechanisms of neuronal protection, anesthesia, pain and depression[53]. Mechano-gated and acid-activated TREK1 and TREK2 are the hypothetical functional homologues of the Aplysia S-type background K^+ channel[53,54]. Recently, genetic inactivation of TREK1 in the mouse has revealed the potential involvement of this K_{2P} channel in a range of neuronal disease states, including pain, ischemia, epilepsy and depression[55-57]. Human TREK1 is highly expressed in the brain, where it is particularly abundant in γ-aminobutyric acid-containing interneurons of the caudate nucleus and putamen[58]. TREK1 is also expressed in the prefrontal cortex, hippocampus, hypothalamus, midbrain serotonergic neurons and sensory neurons of the dorsal root ganglia[55,59-61]. TREK1 is a signal integrator responding to a wide range of physiological and pathological inputs.

Actions of general anesthetics on potassium channels

K_{2P} channels are modulated by a variety of cellular lipids and pharmacological agents, including polyunsaturated fatty acids and volatile general anesthetics. Franks *et al*[62] identified isoflurane-activated a potassium current in specific neurons of the freshwater snail *Lymnaea stagnalis*. This current had the characteristics of a leak or back-

Table 1 The effects of general anesthetics on ion channels

	Volatile anesthetics					Intravenous anesthetics				
	Halothane	Isoflurane	Enflurane	Ether	Ethanol	Propofol	Etomidate	Ketamine	Phentobarbital	Benzodiazepine
GABAA	+	+	+	+	+	+	+	--	+	+
hα1β1	+	+	+							
hα1β2	+	+	+	+	+	+			+	
hα1β1γ2	+	+	+		+	+			+	
hα1β2γ2	+	+	+							
NMDA	--	--	--	--	--			--		
AMPA/Kainate										
GluR1		--	--							
GluR3	--	--	--						--	
GluR2 + 3	--	--	--						--	
GluR6	+	+	+		--	+			--	
K^+										
Kv	--	--	--	--	--					
TREK	+/--	+/--	+/--	+/--	+/--					
HCN	--	--				--		--		
Na^+	--	--	--	--	--	--				

+: Effect of agonist; --: Effect of antagonist; GABAA: γ-aminobutyric acid type A.

ground K^+ channel because it lacked voltage-dependent activation, was non-inactivating and passed currents closely as predicted by the Goldman-Hodgkin-Katz equation for ion conduction through a passive, K^+-selective pore. The *Lymnea stagnalis* IKAn channel, which has biophysical properties very close to the TREK-1 channel, is activated in a range of volatile anesthetic concentrations corresponding to those needed to produce anesthesia in this mollusc[63]. It was therefore important to establish whether the same close relationship between drug efficacy and anesthetic properties would hold true for humans. Most of the experiments have used mouse TREK-1, for which there is abundant biophysical information[54,60]. However, cloned human TREK-1 channel, which like the mouse TREK-1, was also expressed abundantly in brain and had the same biophysical properties and sensitivity to arachidonic acid and polyunsaturated fatty acids. The effect of anesthetics on this channel was examined with exactly the same techniques that were used for the *Lymnea* channel[63]. At half-maximal concentrations of volatile anesthetics used in human general anesthesia[63] (chloroform, 0.79 mmol, halothane, 0.21 mmol, isoflurane, 0.31 mmol), the human TREK-1 channel was markedly activated. Subsequently, a unique family of K subunits with two pore-lining sequences (K_{2P} channels) was discovered that had a wide phylogenetic range and was activated by volatile anesthetics at clinically relevant concentrations[64-66]. Activation of these background K^+ channels in response to volatile anesthetics results in hyperpolarization and silencing of neuronal activity[62,67]. Members of the family can also be activated by xenon[68] and nitrous oxide[69], and differentially activated by isoflurane stereoisomers[70]. C-terminal regions were critical for anesthetic activation in both TASK and TREK channels. Thus both TREK and TASK are possibly important target sites for these agents[64]. Whole-cell patch-clamp experiments showed that chloroform strongly and reversibly activates TREK-1 expression in transfected cells, and this activation was dose dependent, whereas it depressed TASK only slightly and did not affect TRAAK. Chloroform induced a typical TREK-1 background current, characterized by outward rectification that reversed at the predicted value for E_{K+}. Chloroform reversibly and reproducibly hyperpolarized COS cells expressing TREK-1. Both TREK-1 and TASK, but not TRAAK, were opened by halothane. Halothane-induced TASK current had outward rectification and reversed at the predicted value for E_{K+}. The effects of halothane on TASK were rapid and completely reversible. Isoflurane, like halothane, activated both TREK-1 and TASK channels without altering TRAAK conductance. Like chloroform, diethyl ether opened TREK-1 and did not affect TRAAK, whereas it decreased TASK activity.

In excised outside-out patches, activation by volatile anesthetics was not mediated by second-messenger pathways[64]. A 48-pS TREK-1 channel was opened reversibly and in a dose-dependent manner by halothane. No channel activity was observed in the absence of anesthetic, suggesting that halothane converts inactive channels into active ones. The current-voltage (I-V) curve of the chloroform-sensitive current in an outside-out patch showed the outward rectification previously observed in whole-cell recordings. In the inside-out patch configuration, halothane reversibly opened a 12-pS TASK channel. Without anesthetic, a single TASK channel opened and addition of halothane induced the opening of a second channel, which closed again after washout.

Residues in TREK-1 and TASK proteins that are involved in activation by chloroform and halothane were identified using deletions and chimeras. A deletion of the first 42 amino acids in the amino N-terminal region of TREK-1 affected neither anesthetic-induced nor basal channel opening, suggesting that the amino terminus is not important for anesthetic-induced activa-

tion. In contrast, deletion of the last 48 amino acids in the C-terminal region of TREK-1 (TR322) completely suppressed responses to both chloroform and halothane, although it did not affect the basal channel activity. Fusing the C-terminal region of TASK to TR324 did not affect basal activity or restore activation by anesthetics. Further deletion of the C-terminal 72 amino acids in TREK-1 completely abolished both basal and anesthetic-stimulated channel activity. Fusing the C-terminal portion of TASK to TR298 restored basal but not anesthetic-stimulated channel activity. These results demonstrate that anesthetic-mediated TREK-1 opening depends critically on the C-terminal 48 amino acids of the channel[64]. Deletion of the last 147 amino acids in the C-terminal region of TASK did not alter halothane sensitivity, whereas further deletion abolished both basal and halothane induced channel activation. When the C-terminal portion of TREK-1 was fused to TASK, basal but not halothane-induced activity was recovered. These results imply that the region of TASK located between residues 242 and 248 confers sensitivity to halothane[64]. Fusion of the last 48 amino acids of TREK-1 to TASK does not confer sensitivity to chloroform. Moreover, fusion of the last 78 residues of TREK-1 to the anesthetic-resistant channel TRAAK provided no sensitivity to halothane or to chloroform, although the chimera had a prominent basal activity. Introducing the C-terminal portion of TREK-1 has been shown to be essential for both chloroform and halothane. This suggests that the C-terminal region is not the only structural element that confers chloroform sensitivity to TREK-1. Inhalational anesthetics have been proposed to act by binding directly to critical sites on target neuronal proteins[71]. The requirement of segments of the protein sequences situated at the C-terminal of both TREK-1 and TASK for their sensitivity to halothane and chloroform is an indication that anesthetics may bind directly to the channels themselves. However, the possibility remains that these anesthetics bind elsewhere on TASK and TREK-1, and that the identified portions of the C-terminal simply transduce these effects.

Evidence from K_{2P} knock-out mice has further implicated these channels in the mechanism of action of volatile anesthetics. TREK-1 knockout mice were resistant to the effects of volatile anesthetics as determined by the standard MAC assay[57]. Knock-out mice in which other members of K_{2P} channel family have been inactivated (TASK-1 and TASK-3) also show some resistance to the anesthetizing action of volatile anesthetics.

Additional studies with multiple K_{2P} knock-outs will be needed to understand their full importance. K_v channels were first isolated from mutant Drosophila that displayed an abnormal 'shaking' reaction upon exposure to ether[72]. The Shaker phenotype arose from inactivation of a voltage-gated K^+ channel gene, but *in vitro* studies of the effects of anesthetics on this channel family found them to be inhibited at supra-clinical anesthetic concentrations[73,74]. Other potential K^+ channel targets include voltage-gated K^+ channels (K_v) and ATP-activated K^+ channels (K_{ATP}). However, administration of K_{ATP} channel blocking drugs into the neuro-axis did not change isoflurane MAC[75]. Thus, the primary focus of the anesthetic mechanism involving K^+ channels remains on background K^+ channels.

Some potassium channels are known to play beneficial roles in general anesthesia, cardioprotection and neuro-protection. K_{2P} channels are thought to regulate membrane excitability. In CNS, the TREK channels could be activated by membrane stretch, temperature and H^+. It has been shown that TREK channels could be activated by some polyunsaturated fatty acids and volatile general anesthetics which lead to neuro-protective effect. According to a recent study with knockout animals, TREK-1 channels might play an important role in the general anesthetic effect of volatile anesthetics such as halothane, providing an explanation for the neuro-protective effect of general anesthetics.

Potassium channels have been thought to regulate potential in the mitochondrial membrane, respiration rhythmic generation and ion homeostasis. For neuro-protective effects, some potassium channels have been identified in the inner mitochondrial membrane: the K_{ATP} channel, the BK (Ca^{2+}) channel (large conductance Ca^{2+} regulated K^+ channels), the voltage-gated K+ channel 1.3 ($K_{V1.3}$) channel, as well as the TASK-3 channel. It has been demonstrated that potassium influx to the brain mitochondria by the K_{ATP} channel or the BK channel could produce neuro-protective effects on neuron survival under ischemia.

GLUTAMATERGIC ION CHANNELS

Structure and function of glutamatergic ion channels

Glutamate transporters, also called excitatory amino acid transporters, bind and take up extracellular glutamate, a major excitatory neurotransmitter, and regulate glutamatergic neurotransmission in synapses. Glutamatergic neurotransmission can be activated by three distinct families of ligand-gated ion channels: AMPA, kainate and NMDA receptors. Among these ligand-gated ion channels, the NMDA receptor is most important and well-established class.

NMDA is an important chemical molecule (ligand) that selectively acts on the glutamate NMDA receptor (NMDAR). It has been widely demonstrated that NMDARs are important for basic brain function and play a critical role in learning and cognition, memory, and the development of central nervous system hyperactive states. Various chemicals belonging to many drug families have been demonstrated to be NMDAR antagonists.

Actions of general anesthetics on glutamatergic ion channels

Anesthesia, although its exact mechanism is still unclear, is thought to be induced by enhancement of inhibitory neurotransmission or inhibition of excitatory neurotransmission. Block of AMPA receptors can decrease

MAC of halothane by 60%[76,77]. Some gaseous anesthetics such as cyclopropane, xenon and nitrous oxide, as well as the intravenous anesthetic ketamine have been shown to reduce excitatory or glutamate mediated synaptic transmission by blocking NMDA receptors on the postsynaptic membrane[14-18]. In addition, both urethane and enflurane have been found to inhibit excitatory flow in NMDA-expressing Xenopus Oocytes by acting with NMDA receptors[78,79]. Hollmann *et al*[80] demonstrated the reversible dose-dependent inhibition of recombinant NMDA receptors by various volatile anesthetics including isoflurane, sevoflurane and desflurane. These *in vitro* studies indicate a postsynaptic role of glutamate receptors in general anesthesia. Volatile anesthetics may also reduce the excitatory glutamatergic transmission by presynaptic inhibition[81]. However, some studies with knockout mice have failed to find an obvious role of NMDA receptors on anesthetics actions *in vivo*[82-84].

Although MAC is believed to predominantly reflect nocuous reaction at the spinal cord level, results suggests that pharmacologic blockade of glutamatergic neurotransmission is sufficient to result in deep anesthesia. Further, the effect of combinations of NMDA and AMPA receptor antagonists on halothane MAC is consistent with an *in vivo* physiological interaction between the NMDA and AMPA receptors[85]. With the use of the NMDA antagonist magnesium sulfate during general anesthesia for shockwave lithotripsy, a magnesium bolus and infusion can be utilized to reduce analgesic requirements[86].

A theory of anesthesia involving NMDA receptors has been presented, consisting of four hypotheses[87]: (1) The formation of transient higher-order, self-referential mental representations contribute to the states of consciousness. As a result, the brain's representational activity falls below a critical threshold may lead to loss of consciousness; (2) Higher-order mental representations are initiated by neural cell assemblies; (3) the activation of the NMDA receptor channel complex is involved in the formation of such cell assemblies. The activation of this receptor determines the rate at which such assemblies are generated; and (4) Modification of NMDA-dependent processes is the final common pathway of anesthetic effect. Therefore, the agents which directly inactivate the NMDA synapse obviously have anesthetic potential; while the agents that do not directly affect the NMDA synapse will also exert an anesthetic effect if they inhibit NMDA-dependent processes[87]. For example, halothane anesthesia changes the balance between NMDA mediated cholinergic and GABAergic influences on dopamine release and metabolism. Differential sensitivity to halothane of NMDA receptors expressed by the neurons mediating these modulatory influences, or loss of specific NMDA receptor populations through voltage-dependent Mg^{2+} block under anesthesia, could underlie these observations[88].

The molecular action of xenon and isoflurane in inhibiting NMDA receptors occurs by binding to the glycine co-agonist site[89]. This finding may lead to the design of new anaesthetics, as some clinically well-tolerated neuronal protective compounds are also known to bind to this site.

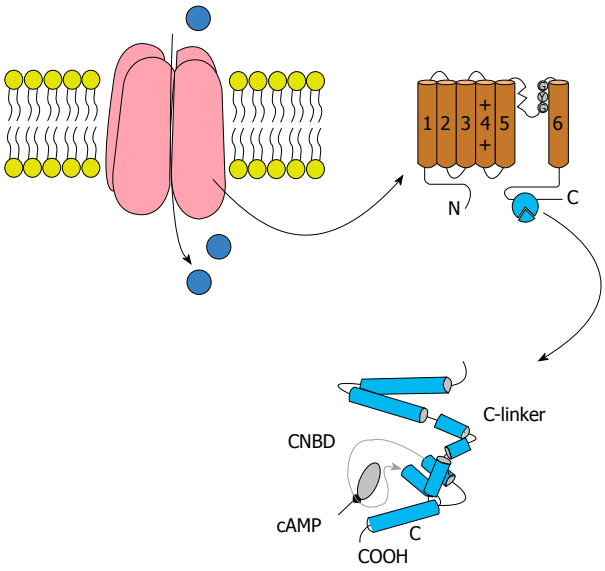

Figure 3 The structure of Hyperpolarization activated cyclic nucleotide channels. Hyperpolarization activated cyclic nucleotide (HCN) channels are made of four subunits. Each subunit contained six trans-membrane segments and S4 acts as the voltage sensor. The pore and filter for ion selection is between S5 and S6. The C-terminal of the HCN channel domain includes the cyclic nucleotide-binding domain (bottom). The domain of the C-linker consists of six a-helices.

HCN CHANNELS

Structure and function of HCN channels

HCN gated channels conduct HCN current (I_f or I_h), that contributes to multiple membrane properties governing cellular excitability[90-92]. Since its first description in 1979[93], extensive work on the I_f current has amply demonstrated its role in the generation and neurotransmitter-induced modulation of pacemaker activity in the heart[94] (Figure 3).

HCN currents are encoded by the four member hyperpolarization activated, cyclic nucleotide-regulated gene family (HCN1-4) with a single channel being composed of a homomeric or heteromeric assembly of four HCN subunits[92]. Cloning of four isoforms of HCN channels in the late 1990s showed their correlation to native HCN channels. HCN channels are unevenly distributed on the cell membrane; for example, HCN1 is preferentially expressed on distal dendritic membranes of pyramidal cells in the cortex and hippocampus. Comparison of the properties of native pacemaker channels with those of HCN channels has provided information concerning the composition and molecular features of native channels in different cardiac regions. In addition, HCN channels conduct a cationic current I_f that contributes to autorhythmicity in both the brain and heart. Consistently, dendritic I_h current density and amplitude increases as

one moves farther away from the soma[95-98]. Dendritic I_h normalizes temporal summation[95,97,99-100], disconnects somatic and dendritic spike initiation zones[97], and probably limits the development of long-term potential[101]. For example, dendritic expression of HCN1 normalizes somatic voltage responses and spike output in hippocampal and cortical neurons. It was reported previously that HCN2 is predominantly expressed in dendritic spines in reticular thalamic nucleus (RTN) neurons, but the functional impact of HCN2 expression remains unknown.

HCN2 and HCN4 are the two major isoforms present in the thalamic RTN[102], but the relative contribution of the two isoforms to I_h in RTN neurons is unknown. Somatic I_h in RTN neurons is small even at hyperpolarized membrane potentials[103,104]. HCN2 is the major isoform generating I_h in RTN because HCN2 deletion abolishes I_h and reproduces the effects of the HCN channel blocker ZD7288 (4-ethylphenylamino-1, 2-dimethyl-6- methylamino- pyrimidinium chloride). Functional expression of HCN2 in RTN constrains intrinsic excitability and ionotropic glutamate receptor-mediated synaptic integration, thereby reducing spike-dependent GABAergic output. Co-localization of HCN2 channels and the AMPA receptor GluR4 subunit is evident in the spines of RTN neurons, thus providing a structural basis for an interaction between intrinsic and synaptic conductance[105].

The relevance of I_f to pacemaker generation and modulation makes channels a natural target for drugs aiming to control heart rate pharmacologically. Agents which act by selective inhibition of I_f have been developed to reduce heart rate, and these drugs have a high potential for treatment of diseases where heart rate reduction is beneficial, such as angina and heart failure. Devices which are able to replace electronic pacemakers and are based on the delivery of a cellular source of pacemaker channels to non-pacing tissue (biological pacemakers) are likely to be developed in the near future for use in therapies for diseases of heart rhythm[106].

Actions of general anesthetics on HCN channels

In the central nervous system, the inhibition of HCN channels by general anesthetics has been suggested to contribute to their anesthesia actions. Inhibition of homomeric HCN1 channels is mediated by anesthetic association with the membrane embedded channel core, a domain that is highly conserved between this isoform and the relatively insensitive HCN2 and 4 subunits. Modeling of the equilibrium and kinetic behavior of HCN1 channels in the absence and presence of anesthetic reveals that gating is best described by models wherein closed and open states communicate by a voltage-independent reaction with no significant equilibrium occupancy of a deactivated open state at non-permissive voltages. Propofol modifies gating by preferentially associating with closed-resting and closed-activated states but a low affinity interaction with the activated open state shapes the effect of the drug under physiological conditions. The mechanism of HCN channel gating provides a framework that will facilitate development of propofol derivates that have altered pharmacological properties and therapeutic potentials.

Activation of native I_f pacemaker channels and channels formed on heterologous expression of some isoforms of their pore forming HCN subunits, is inhibited by the intravenous general anesthetic propofol (2, 6-diisopropylphenol). Decoupling of HCN channel gating from cAMP and internal protons reveals that changes in these second messengers are neither necessary nor sufficient to account for the actions of propofol. Thus, propofol slows and hyperpolarizes activation of HCN1 channels but it has only weak or no effect on HCN2 and HCN4[107-109] whereas halothane hyperpolarizes HCN1 but suppresses that maximal current carried by HCN2 channels[110,111]. The voltage dependence of I_f activation is regulated by cAMP[92], internal protons (H^+)[112] and several signaling lipids[113-115]. The molecular basis by which lipid messengers alter channel function have not been established. Interestingly, in the case of halothane, HCN isoform selectivity is dependent on the activation status of the cAMP gating ring such that the responses of HCN1 and HCN2 channels are essentially identical when cAMP levels are high or the inhibitory effects of the gating ring are eliminated by deletion[116]. Studies on the effects of propofol on recombinant HCN1, HCN2, and HCN4 channels found that the drug inhibits and slows activation of all three channels at clinically relevant concentrations. In Oocytes expression studies, HCN1 channel activation was most sensitive to slowing by propofol. HCN1 channels also showed a marked hyperpolarizing shift, induced by propofol, in the voltage dependence of activation and accelerated deactivation. Furthermore, propofol reduced heart rate in an isolated guinea pig heart preparation over the same range of concentrations. These data suggest that propofol modulation of HCN channel gating is an important molecular mechanism that can contribute to the depression of central nervous system function and also lead to bradyarrhythmias in patients receiving propofol during surgical anesthesia.

Conventional HCN1 knockout mice were used to test directly the contributions of specific HCN subunits to the effects of isoflurane, an inhalational anesthetic, on membrane and integrative properties of motor and cortical pyramidal neurons *in vitro*. Compared with wild-type mice, residual I_f from knockout animals was smaller in amplitude and presented with HCN2-like properties. Isoflurane increased temporal summation of excitatory postsynaptic potentials (EPSPs) in cortical neurons from wild-type mice, an effect predicted by simulation of anesthetic-induced dendritic I_f inhibition. Accordingly, anesthetic-induced EPSP summation was not observed in cortical cells from HCN1 knockout mice. In wild-type mice, the enhanced synaptic summation observed with low concentrations of isoflurane contributed to a net increase in cortical neuron excitability. HCN channel subunits have been shown to account for distinct

anesthetic effects on neuronal membrane properties and synaptic integration. Inhibition of HCN1 by anesthetics in cortical neurons has been shown to contribute to the synaptically-mediated slow-wave cortical synchronization that accompanies anesthetic-induced hypnosis[111].

Na^+ CHANNELS AND GENERAL ANESTHESIA

Structure and function of Na^+ channels

Voltage-gated Na^+ channels have received short shrift as possible anesthetic targets, mainly because early reports failed to demonstrate their significant effects in myelinated axons. However, recently a variety of evidence supports a role for sodium channels in general anesthesia.

The sodium channel family has nine homologous pore-forming α-subunits and these subunits show distinct cellular and sub-cellular distribution, depending on different species and tissues[117]. The pore forming component of sodium channels is a 260 kDa glycoprotein a-subunit, with large intracellular N- and C-terminal. Four internally homologous repeated domains are contained in this subunit (I -IV) and over 50% of the sequence of these domains has been identified. It has been demonstrated that six segments (S1-S6) are contained in each domain and that they form transmembrane α-helices. In addition, four integral membrane glycoprotein subunits have been identified. Generally, the α-subunit is sufficient for the basic functions of sodium channels while expression of β-subunits regulates inactivation and shifts voltage dependence in the direction of more negative potentials. Their modular structures allow interactions between multiple regions of the channel to regulate gating, rapid channel opening and closure.

Functional domains of sodium channels have been identified by many potent toxins[118]. For example, the dinoflagellate toxin (saxitoxin) and the puffer fish poison (tetrodotoxin, TTX) bind to the α-subunit of sodium channel on an extracellular site. For TTX-sensitive sodium channels, Na^+ permeability is strongly blocked by these toxins with high potency. In contrast, for TTX-insensitive sodium channels, it is evident that the affinity of TTX to these channels is 200-fold lower. Some lipid soluble steroids, such as veratridine and the frog skin toxin (batrachotoxin) as well as the plant alkaloids (aconitine), bind to the α-subunit of sodium channels on another extracellular site. With a high affinity for the open state of sodium channels, these steroids slow inactivation of sodium channels, resulting in an agonist effect to the ion channels.

Actions of general anesthetics on sodium channels

Voltage-gated sodium channels are regulated by the membrane potential and lead to the passive flux of Na^+ into or out of the cell. In most excitable cells and tissues such as nerve, muscle and heart, voltage-gated sodium channels account for the rapid depolarization of action potential[117]. The pharmacological profile as well as ion selectivity of the sodium channel has been explained by a dynamic model of receptor gating. As described by modulated receptor gating, a variety of drugs, such as local anesthetics, class I anti-arrhythmic drugs, and class I antiepileptic drugs, have shown voltage-dependent and frequency-dependent block of sodium channels. According to this model, these properties are conferred by different drug affinities for the various functional states of the channel (resting, open, inactivated). The evidence that mammalian voltage-gated sodium channels are sensitive to general anesthetics at clinically relevant concentrations comes from careful analysis of anesthetic effects on heterologously expressed sodium channels. It has been demonstrated that one neuronal isoform (Nav1.2) is inhibited by various potent volatile anesthetics by a voltage-independent block of peak current and a hyperpolarizing shift in the steady-state inactivation[119]. In addition, many volatile anesthetics, especially isoflurane, have been demonstrated to inhibit multiple mammalian sodium channel isoforms[120] including $Na_v1.2$[119], $Na_v1.4$ and $Na_v1.6$[121,122], $Na_v1.5$[123], and $Na_v1.8$. Although early studies suggested that the peripheral tetrodotoxin-resistant isoform $Na_v1.8$, expressed in amphibian Oocytes, was resistant to inhaled anesthetics[124], more focused reports in neurons indicate that $Na_v1.8$ is significantly inhibited by isoflurane at concentrations similar to those that inhibit most other isoforms[125]. Potent volatile anesthetics also inhibit native Na^+ channels in isolated nerve terminals[122,126] as well as dorsal root ganglion neurons[127]. In contrast, xenon has been found to have no obvious effect on Na^+, Ca^{2+}, or K^+ channels in isolated cardiomyocytes[128]. However, recent studies suggest that xenon can in fact block neuronal sodium channels at clinically relevant concentrations.

Generally, two principal mechanisms contribute to the inhibition of Na^+ channel by volatile anesthetics. These are voltage-independent block of peak currents and enhanced inactivation due to a hyperpolarizing shift in the voltage dependence of steady-state fast inactivation. There are significant differences between isoforms in the contributions of each mechanism to overall inhibition[120,127]. Volatile anesthetics, but not non-immobilizers, also inhibit native neuronal and nerve terminal Na^+ channels, supporting the notion that depression of synaptic neurotransmitter release occurs by Na^+ channel blocking[120,127]. A recent study demonstrated that NaChBac, a prokaryotic homologue of voltage-gated Na^+ channels, is also inhibited by volatile anesthetics[129]. Anesthetic interactions with NaChBac might ultimately allow co-crystallization with anesthetic for three-dimensional structure determinations by X-ray crystallography, as achieved for voltage-gated K^+ channels, to determine the site of interaction of anesthetics with a voltage-gated ion channel. It is also intriguing that the binding sites for anesthetics on ion channels exist in prokaryotic homologues, indicating a remarkable evolutionary conservation.

Voltage-gated Na^+ channels have been demonstrated to be insensitive to general anesthetics in early studies on myelinated axons. However, smaller diameter unmyelinat-

ed fibers and nerve terminals are found to be sensitive to Na^+ channel block and do not possess the considerable reserve of conduction seen in myelinated nerves. Many studies summarized earlier demonstrate that inhaled anesthetics partially impair Na^+ channel function at MAC (minimum alveolar concentration). Moreover, a variety of evidence supports a role for sodium channels in general anesthesia *in vivo*, for example the increase in cerebrospinal fluid Na^+ concentration increases MAC of halothane (equivalent to ED_{50}) in rats[130]. Intravenous administration of the Na^+ channel blocker lidocaine reduces MAC for several volatile anesthetics in rats[131], and intravenous or intrathecal infusions of riluzole, a potent inhibitor of Na^+ channels and glutamate release, decrease isoflurane MAC in rats[132]. Finally, intrathecal but not intraventricular administration of veratridine, a toxin that maintains Na^+ channels in their open state, increases the MAC for isoflurane in rats. Collectively, these results point to anesthetic inhibition of Na^+ channels as a plausible mechanism for the mediation of immobility produced by inhaled anesthetics.

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February 26-29, 2012
11th International Dead Sea Symposium on Cardiac Arrhythmias and Device Therapy
International Convention Center,
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March 2-3, 2012
Twelfth Annual John M Templeton Jr Pediatric Trauma Symposium
Philadelphia, PA, United States

March 25-30, 2012
5th World Congress of Anaesthesiologists
Buenos Aires, Argentina

April 11-13, 2012
Society of Trauma Nurses 2012 Annual Conference
Savannah, GA, United States

May 3-5, 2012
18th Annual Spring Meeting of the Anesthesia History Association
Kansas City, MI, United States

May 10-11, 2012
National Trauma Institute 2012 Annual Conference
San Antonio, TX, United States

May 18-23, 2012
American Thoracic Society 2012 International Conference
San Francisco, CA, United States

May 24-25, 2012
European Society of Intensive Care Medicine Summer Conference: Trauma Update 2012
The Royal Society,
London, United Kingdom

May 26-29, 2012
10th World Congress for Nurse Anesthetists
Ljubljana, Slovenia

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Omni Shoreham Hotel,
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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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