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**T-cell ageing in end-stage renal disease patients: Assessment and clinical relevance**

Meijers RWJ *et al.* T-cell ageing in end-stage renal disease patients

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**Abstract**

End-stage renal disease (ESRD) patients have a defective T-cell-mediated immune system which is related to excessive premature ageing of the T-cell compartment. This is likely to be caused by the uremia-associated pro-inflammatory milieu, created by loss of renal function. Therefore, ESRD patients are highly susceptible for infections, have an increased risk for virus-associated cancers, respond poorly to vaccination and have an increased risk for atherosclerotic diseases. Three ageing parameters can be used to assess an immunological T-cell age. First, thymic output can be determined by assessing the T-cell receptor excision circles-content together with CD3 expression within the naïve T cells. Second, the telomere length of T cells and third the T-cell differentiation status are also indicators of T-cell ageing. Analyses based on these parameters in ESRD patients revealed that the immunological T-cell age is increased by on average 20 years compared to the chronological age. After kidney transplantation (KTx) the aged T-cell phenotype persists although the pro-inflammatory milieu is diminished. This might be explained by epigenetic modifications at hematopoietic stem cells level. Assessment of an immunological T-cell age could be an important tool to identify KTx recipients who are at risk for allograft rejection or to prevent over-immunosuppression.

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**Key words:** End-stage renal disease patients; Kidney transplantation; T-cell ageing; T-cell differentiation; Uremia

**Core tip:** The uremia-induced inflammatory environment in end-stage renal disease (ESRD) patients is associated with a prematurely aged T-cell compartment, resulting in defective T-cell-mediated immunity. ESRD patients are highly susceptible for infections, have an increased risk for virus-associated cancers, respond poorly to vaccination and have an increased risk for atherosclerotic diseases. Adequate renal replacement therapy in the form of kidney transplantation is able to diminish the uremic pro-inflammatory environment but unsuccessfully reverses the aged T-cell system. Assessment of T-cell ageing might be a tool to facilitate individualization of immunosuppressive regimes and prevent over-immunosuppression and its associated clinical complications in kidney transplant recipients.

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**INTRODUCTION**

Loss of renal function is strongly associated with a defective immune system which is known as uremia-associated immune deficiency[[1-3](#_ENREF_1)]. Retention of uremic molecules and cytokines in end-stage renal disease (ESRD) patients are key mechanisms in generating oxidative stress and inflammation[[2](#_ENREF_2),[4](#_ENREF_4),[5](#_ENREF_5)]. This creates a pro-inflammatory environment in which both the innate (first line of defense, a-specific)[[3](#_ENREF_3),[6-8](#_ENREF_6)] as well as the adaptive (specific) immune system are affected (Figure 1)[[3](#_ENREF_3),[9](#_ENREF_9),[10](#_ENREF_10)].

T cells, members of the adaptive immune system, are the best-studied immune cells in ESRD patients and in the field of transplantation they are the main target of immunosuppressive medication[[11](#_ENREF_11)]. The uremia-associated pro-inflammatory milieu causes T-cell defects associated with premature T-cell ageing when compared to healthy age-matched individuals (Figure 1)[[12](#_ENREF_12)]. Analysis of the T-cell compartment in ESRD patients revealed that the immunological age of T cells is increased by 20 years compared to their chronological age (Figure 1)[[12](#_ENREF_12)].

The dysfunctional immune system of ESRD patients has a substantial clinical impact on both the morbidity and mortality of ESRD patients. Patients are highly susceptible for infections[[13](#_ENREF_13),[14](#_ENREF_14)], have an increased risk for virus-associated cancers[[15](#_ENREF_15)], respond poorly to vaccination[[16](#_ENREF_16)] and have an increased risk for atherosclerotic diseases[[17](#_ENREF_17),[18](#_ENREF_18)].

In this review, the concept of uremia-associated age-related changes of T cells is highlighted focusing on the assessment of an immunological T-cell age, clinical implications and possible therapeutic options for ESRD patients.

**CONCEPT OF T-CELL AGEING**

With normal healthy ageing, the T-cell immune system ages as well[[19](#_ENREF_19)]. Hematopoietic stem cells (HSCs), generated in the bone marrow, give rise to myeloid as well as lymphoid progenitor cells[[20](#_ENREF_20)]. T cells are generated from the latter. With increasing age, HSCs are skewed towards myeloid-generating subsets at the expense of lymphoid-generating HSCs, resulting in a lower number of progenitor T cells. These progenitor T cells are further “educated” in the thymus in which naïve T cells will form specific receptors on their cell surface known as T-cell receptors (TCRs). With increasing age, the thymus involutes[[21](#_ENREF_21),[22](#_ENREF_22)]. This process involves a decrease in tissue in combination with a loss of tissue organization with the net outcome that numbers of naïve T cells leaving the thymus, known as recent thymic emigrants (RTEs) are reduced. Involution of the thymus starts at birth and is accelerated during adolescence[[23](#_ENREF_23)].

This explains the lymphopenic number in naive T cells with increasing age. Despite the fact that the naïve T-cell pool can also be maintained by homeostatic proliferation in which TCR triggering in combination with the cytokines Interleukin (IL)-7 and IL-15 expand T cells[[24](#_ENREF_24)], the net effect is a diminished number of naïve T cells and the number of memory T cells in the peripheral blood of elderly individuals is preserved[[25](#_ENREF_25)]. A relatively expanded number of naïve T cells by homeostatic proliferation results in a T-cell pool with a restricted TCR repertoire[[24](#_ENREF_24),[26](#_ENREF_26)]. A diverse TCR repertoire is a necessary prerequisite for an adequate and effective T-cell response towards newly encountered antigens[[27](#_ENREF_27)].

After encountering and activation by an antigen, a naïve T cell will proliferate and become a memory T cell. During physiological ageing the population of antigen-experienced memory T cells will increase and the majority of these cells will become highly differentiated. These cells are known to have an increase in pro-apoptotic markers[[28](#_ENREF_28)] and loss in co-stimulatory molecule CD28[[29](#_ENREF_29),[30](#_ENREF_30)]. CD28 plays an important role in the activation of T cells and a loss of CD28 can result in insufficient activation, shorter replicative lifespan and a higher toxicity[[29](#_ENREF_29)]. Furthermore, highly differentiated cells are known to have a reduction in their telomere length[[31](#_ENREF_31)].

A telomere is a region of repetitive [nucleotide](http://en.wikipedia.org/wiki/Nucleotide)s which is located at the end of each chromosome and prevents chromosomal instability. Loss of telomere length has been linked to an increased risk for tumor development and to T-cell ageing[[32](#_ENREF_32),[33](#_ENREF_33)].

**ASSESSING AN IMMUNOLOGICAL T-CELL AGE**

A global assessment of the immunological age of the T-cell system can be performed by the analysis of three ageing parameters. During the formation of the T-cell receptor (TCR) in the thymus, DNA sequences in the TCR loci are deleted and circularized into episomal DNA molecules, so called single joint TCR excision circles (TREC), a process known as TCR rearrangement[[34](#_ENREF_34)]. This TREC remains in the newly formed naïve T cells leaving the thymus. Upon replication of these cells in the periphery, the TREC is only transferred to one daughter cell resulting in a reduction of TRECs in the naïve daughter T cells. With an increasing age, the number of RTEs containing a TREC declines log linearity due to a lower thymic output of RTEs and an increase in proliferation of naïve T cells. The TREC content can be determined using a quantitative polymerase chain reaction (qPCR) method normalized to the single-copy albumin gene[[34](#_ENREF_34),[35](#_ENREF_35)]. Next to the TREC content, these RTEs can be detected by measuring the expression of CD31 within the naïve T-cell pool[[36](#_ENREF_36),[37](#_ENREF_37)]. In addition to the thymic output of T cells, the diversity of the TCR repertoire can be analyzed by sequencing in order to determine the loss of TCR specificities within the T-cell population and to assess the percentage of oligoclonal T cells[[27](#_ENREF_27),[38](#_ENREF_38)]. Recently, a novel TREC assay in which the TCR diversity was combined with the TREC content to get quantitative insight into intra-thymic and post-thymic proliferative capacity of T cells and its alterations upon ageing[[39](#_ENREF_39)].

As a second parameter for the assessment of an immunological T-cell age, the T-cell telomere length can be determined as a measurement for the proliferative history of a T-cell population[[40](#_ENREF_40)]. A decline in telomere length is highly associated with an increased proliferative history. A commonly used method to assess a relative telomere length (RTL) is the fluorescent *in situ* hybridization (FISH) method[[41](#_ENREF_41),[42](#_ENREF_42)]. During this procedure a labeled peptide nucleic acid (PNA) probe binds to the telomere repeats which can be read-out by fluorescent microscopy or by fluorescence measurements using a flow cytometry (flow FISH). The RTL can be calculated by relating the intensity of the bound PNA probe to that of a T-cell lymphoblastic leukemia (1301 CCRF-CEM) cell-line, known for its long telomeres, as an internal control[[41](#_ENREF_41)]. Inclusion of antibodies in this method makes it possible to analyze the telomere length in different T-cell populations (*i.e.,* CD4+ and CD8+ T cells)[[2](#_ENREF_2),[41](#_ENREF_41)]. A limitation of this assay is the temperature (82 ⁰C) which is required for DNA annealing which makes the use of stable fluorochromes necessary[[41](#_ENREF_41),[42](#_ENREF_42)]. Quantum dots (nanoparticles) were found to be highly fluorescent, bind to antibodies and have much better temperature stability. Quantum dots conjugated with antibodies directed to T-cell antigens were found to retain most of their fluorescence following the annealing step. The use of quantum dots can be a solution for the limitations in antibody use in the flow-FISH procedure and allows to assess a telomere length in different T-cell subsets within one assay[[42](#_ENREF_42)].

In addition to the telomere length, the activity of the telomerase can be measured. Telomerase is responsible for maintaining telomere length and the cellular replicative potential and an impaired activity of telomerase results attrition of telomeres[[19](#_ENREF_19)]. Measuring the activity of telomerase gives additional information on the telomere shortening. This assay is based on the capacity of a test sample to amplify a telomere template[[43](#_ENREF_43)].

The differentiation status of the T-cell compartment can be used as a third parameter to assess an immunological age. The increase in highly differentiated memory cells with increasing age can be determined by analysis of the phenotype of circulating T-cells using multicolor flowcytometry. Based on the expression of the chemokine (C-C motif) receptor 7 (CCR7), enabling cells to migrate to secondary lymphoid organs, and CD45RO, an isoform of the leukocyte common antigen expressed on memory T cells, a distinction within the memory T-cell compartment can be made. The different memory T cell subsets include Central Memory (CM) (CCR7+ and CD45RO+), able to home to secondary lymph nodes and producing mainly IL-2 which is necessary for the proliferation of T cells, Effector Memory (EM) (CCR7- and CD45RO+), able to migrate to peripheral tissues exerting direct effector functions and terminally differentiated effector memory CD45RA+ (EMRA) (CCR7- and CD45RO-), which exert cytotoxic activities and are highly susceptibility to apoptosis[[44](#_ENREF_44)]. Moreover, these terminally differentiated cells often lose the expression CD28 which makes them less dependent on co-stimulation to become activated[[45](#_ENREF_45)]. In addition, CD57 can be measured as a marker for highly differentiated memory T cells[[12](#_ENREF_12),[46](#_ENREF_46)]. CD95 (FAS) and CD279 (known as programmed death receptor-1 (PD-1)) are both commonly used as pro-apoptotic markers[[12](#_ENREF_12),[28](#_ENREF_28),[47](#_ENREF_47)].

**THE AGED T-CELL SYSTEM IN ESRD PATIENTS**

Based on the analyses of the T-cell ageing parameters, *i.e*., assessment of TREC- content, relative telomere length and differentiation status we showed that the immunological age of ESRD patients is advanced by 20 years compared to their calendar age[[12](#_ENREF_12)]. As compared to an age-matched healthy control, ESRD patients had a lower thymic output of naïve T cells, a decline in the T-cell telomere length and an increase in the differentiation status towards the terminally differentiated memory phenotype with a large number of CD28-negative (or CD28null) T cells (Figure 1)[[12](#_ENREF_12)]. Progressive loss of renal function was highly correlated with a lack of IL-7, a loss of naïve T cells and an increase in terminally differentiated CD8+ T cells[[48](#_ENREF_48)]. The effects of renal replacement therapy (RRT) on the T-cell ageing parameters seemed to be small and were limited to the CD8+ T-cell compartment of young ESRD patients[[2](#_ENREF_2)]. The type of RRT did not influence the ageing parameters since both hemodialysis (HD) and peritoneal dialysis (PD) patients showed signs of an aged T-cell compartment[[2](#_ENREF_2)]. Moreover, the duration of dialysis did not seem to influence the ageing parameters[[49](#_ENREF_49)]. Furthermore, the type of underlying kidney disease was not related to any parameter of immunological ageing[[2](#_ENREF_2)] indicating that the loss of renal function is the dominant factor for a decreased thymic output of naïve T cells and increased differentiation/proliferation of memory T cells.

Cytomegalovirus (CMV) is known to affect the T-cell compartment which closely resembles ageing[[46](#_ENREF_46),[50-52](#_ENREF_50)]. Infection with the virus results in chronic latency and the effects of CMV on the T-cell compartment are relevant, since approximately 70% of the ESRD patients is infected with CMV[[50](#_ENREF_50)]. In these patients, CMV was associated with an increased number of highly differentiated CD4+ and CD8+ T cells and a relatively small decline in CD8+ T-cell telomere length[[46](#_ENREF_46),[50](#_ENREF_50),[53](#_ENREF_53)]. The effects were restricted to the memory T-cell compartment since the thymic output of T cells was not affected. Therefore we concluded that CMV only affects the differentiation status of circulating T cells[[46](#_ENREF_46),[50](#_ENREF_50),[53](#_ENREF_53)].

**CLINICAL IMPLICATIONS OF AN AGED T-CELL COMPARTMENT**

The uremia-associated prematurely aged T-cell immune system has a substantial clinical impact leading to an increased morbidity and mortality. ESRD patients are highly susceptible for infections which might further contribute to the pro-inflammatory milieu. For instance periodontitis, which is common in patients with chronic kidney disease (CKD), often leads to inflammation[[54](#_ENREF_54)].

T cells of ESRD patients have an impaired production of IL-2 and the inadequate T-cell proliferative capacity results insufficient T-cell responses[[55-57](#_ENREF_55)]. This in combination with low numbers of T cells results into inadequate T-cell responses directed to viruses and a decreased tumor surveillance which significantly increases the risk for virus-associated tumors[[15](#_ENREF_15),[58](#_ENREF_58)]. Next to IL-2, in hemodialysis (HD) patients it was found that activated T cells have impaired responses to tumor necrosis factor (TNF)-α, implying a state of tachyphylaxis[[59](#_ENREF_59)].

Following vaccination against hepatitis B, the formation of antigen-specific CD4+ EM T cells is severely impaired in ESRD patients[[56](#_ENREF_56)]. The poor development of IL-2 producing CD4+ EM T cells in patients with ESRD was strongly associated with a low generation of antibodies towards hepatitis B antigens[[56](#_ENREF_56)]. The inability to maintain protective antibody titers after T-cell dependent vaccinations[[60](#_ENREF_60),[61](#_ENREF_61)] or after a natural infection[[62](#_ENREF_62),[63](#_ENREF_63)] might be caused by a loss of antigen-specific T cells as a result of their increased susceptibility for apoptosis[[12](#_ENREF_12),[47](#_ENREF_47)].

Furthermore, the loss in TCR diversity of naïve T cells due to a lower number of RTEs but an increase in proliferated naïve T cells is linked to a decreased efficiency of vaccination but also to an increased susceptibility for infections and cancers[[26](#_ENREF_26),[64](#_ENREF_64)].

CD4+ T cells lacking CD28 expression, are found to be highly cytotoxic as they produce large amounts of interferon (IFN)-γ and TNF-α and release granzyme-B and perforin upon activation. In several studies[[17](#_ENREF_17),[65](#_ENREF_65)] it is shown that these cytotoxic cells are present in unstable atherosclerotic plaques and are associated with an increased risk for recurrence of both acute coronary events and ischemic stroke resulting in a higher mortality rate[[66](#_ENREF_66)]. As confirmed in ESRD patients, high numbers CD4+CD28null T cells is strongly associated with a history of cardiovascular diseases[[17](#_ENREF_17),[18](#_ENREF_18),[65](#_ENREF_65),[67](#_ENREF_67)].

CD8+CD28null T cells contain a subpopulation of cells possessing immunosuppressive capacities[[68](#_ENREF_68),[69](#_ENREF_69)] and has therefore been linked to a decreased vaccination responsiveness of healthy individuals[[70](#_ENREF_70)]. These immunosuppressive capacities also suggest that these cells could be important in preventing allograft rejection after kidney transplantation (KTx). Indeed, we recently demonstrated that patients with an expanded population of highly differentiated (EMRA) CD8+CD28null T cells had a lower risk for allograft rejection after KTx[[71](#_ENREF_71)]. Another explanations might be that CD8+CD28null T cells represents clonal expansions of particular antigen-specific CD8+ T cells that compete for immunologic space which is associated with reduction of T-cell diversity[[72](#_ENREF_72)]. This might affect the diversity of alloreactive T-cells as well. Next to these highly differentiated CD8+ T cells in KTx recipients, a high proportion of highly differentiated CD4+ T cells was also linked to a lower risk for allograft rejection[[73](#_ENREF_73)].

**PREMATURE T-CELL AGEING AND KIDNEY TRANSPLANTATION**

After KTx, the levels of pro-inflammatory proteins and oxidative stress decrease rapidly to levels that are comparable to healthy individuals[[74](#_ENREF_74)]. Despite this, the uremia- associated prematurely aged T-cell immune system existed after KTx. *(Meijers, R.W.J. et al. 2014 submitted)*

Immunosuppressive treatment affected the number of highly differentiated cells directly post-KTx. However after tapering the immunosuppressive medication, these highly differentiated T-cell numbers were restored to pre-KTx values. Furthermore, the telomere length of the T-cell compartment did not change and thymic function was not improved the first year post-KTx *(Meijers, R.W.J. et al. 2014 submitted)*. Even after T-cell depleting immunosuppressive therapy (i.e. rabbit antithymocyte globulin (rATG)) T cells are repopulating by homeostatic proliferation instead of a higher thymic output of naïve T cells[[75](#_ENREF_75),[76](#_ENREF_76)]. Therefore, the uremia-associated immunological ageing seems stably imprinted in the T-cell system and not reversible by KTx.

Normal ageing is associated with, epigenetic changes in HSCs resulting in a shift in the balance towards myeloid precursors at the expense of the lymphoid ones[[77](#_ENREF_77),[78](#_ENREF_78)]. Healthy ageing results in genetic alterations affecting T cells at developmental stages leading to phenotypic as well as functional changes[[79](#_ENREF_79)]. In ESRD patients, uremia is able to cause epigenetic changes[[80](#_ENREF_80)]. Young *et al*[[81](#_ENREF_81)] 2012 found that methylation of the KLOTHO gene is initiated by oxidative stress in ESRD patients. KLOTHO deficient mice created a syndrome that resembles human ageing[[82](#_ENREF_82)]. Although KTx reverses the uremic proinflammatory environment[[74](#_ENREF_74)] it is unable to induce changes at the epigenetic level. The persistence of the aged T-cell phenotype post-KTx has several clinical implications as it may increase the risk for infections, malignancies and cardiovascular diseases in KTx recipients. T-cell lymphopenia has been associated with a high risk for infections and malignancies post-KTx[[83](#_ENREF_83),[84](#_ENREF_84)].

Due to ageing of the T-cell compartment, elderly patients are more vulnerable for drugs toxicity, infections and malignancies caused by over-immunosuppression. In these patients, the incidence of virus-associated cancers is even higher post-KTx as it is pre-KTx[[58](#_ENREF_58),[85](#_ENREF_85)]. Over-immunosuppression might be prevented after mapping the T-cell immune system of the transplant recipient[[73](#_ENREF_73),[86](#_ENREF_86)] as T cells are the main target of immunosuppressive medication[[11](#_ENREF_11)]. A study of Ducloux *et al*[[87](#_ENREF_87)] in2010 showed that prolonged CD4+ T-cell lymphopenia after severe T-cell depletion by rATG is associated with an increased risk for infections and mortality post-KTx. High TREC values implying for a “younger” T-cell compartment pre-KTx, is associated with a better reconstitution of T-cell numbers after rATG and lower risk for infections and cancer post-KTx[[87](#_ENREF_87)].

**THERAPEUTIC OPTIONS TO REVERSE T-CELL AGEING**

As mentioned earlier, RRT did not reduce T-cell ageing since no major differences between patients on dialysis and predialysis patients with respect to the T-cell ageing parameters were observed[[2](#_ENREF_2)]. Adequately targeting the presence of the pro-inflammatory environment in ESRD patients by KTx[[74](#_ENREF_74)] did not successfully reverse the aged T-cell immune system.

Another method to reduce the level of oxidative stress and inflammation in ESRD patients is targeting the transcription factor Nuclear factor-erythroid-2-related factor 2 (Nrf2) which is an important regulator of genes encoding antioxidant and detoxifying molecules[[88](#_ENREF_88)]. Treatment with bardoxolone methyl, which is an activator of Nrf2 may attenuate T-cell ageing in ESRD patients[[88](#_ENREF_88)]. However, treatment is restricted due to the increased risk of cardiovascular diseases after treatment with bardoxolone[[89](#_ENREF_89)].

Another therapeutic option that might be able to improve T-cell function in ESRD patient is treatment with IL-7, a key cytokine for homeostatic proliferation of naïve T cells, that is reduced in patients causing a depletion of naïve T-cell pool[[48](#_ENREF_48),[90](#_ENREF_90)]. The first human studies, in which IL-7 was administered, are promising since an increased naïve T-cell pool with a broader TCR repertoire diversity was found[[38](#_ENREF_38),[91](#_ENREF_91)]. At present, IL-7 administration has not been tested in patients with ESRD.

**CONCLUSION**

Progressive loss of renal function creates a pro-inflammatory milieu which is highly associated with a dysfunctional immune system. This is a logical explanation for the increased vulnerability for infections, poor vaccination responses, high risk for malignancies and high risk for atherosclerotic diseases. Analysis of the T-cell system showed that ESRD patients have a prematurely aged T-cell compartment resulting in an impaired function. ESRD patients have a lower thymic output of naïve T cells, T cells have shorter telomeres and the T-cell compartment is shifted towards more differentiated T cells.

Therapeutic options to minimize morbidity and decrease mortality by improving or even fully reversing the aged T-cell phenotype are warranted. Although improvement of renal function by adequate renal replacement therapy in the form of KTx, which drastically decreases the uremia-associated pro-inflammatory milieu, the prematurely aged T-cell phenotype appeared to be irreversible. Therefore the aged T-cell immune system remains an important determinant of the dysfunctional immune system post-KTx. More research is necessary to fully understand the uremia-associated premature T-cell ageing phenomenon, also at earlier developmental stages of T-cells, to be able to successfully intervene and increase the life-span of ESRD patients.

Until today, all KTx recipients receive the same standard immunosuppressive therapy to prevent allograft rejection. Recently it was shown that the effect of calcineurin-inhibitors and rapamycin on peripheral blood mononuclear cells (PBMCs) was different between young and elderly individuals[[92](#_ENREF_92)]. Assessing an immunological T-cell age using T-cell ageing parameters as described in this review, may guide clinicians in decision-making with respect to transplanting an ESRD patient or not, adjusting immunosuppression following KTx to minimize its long-term-associated adverse events.

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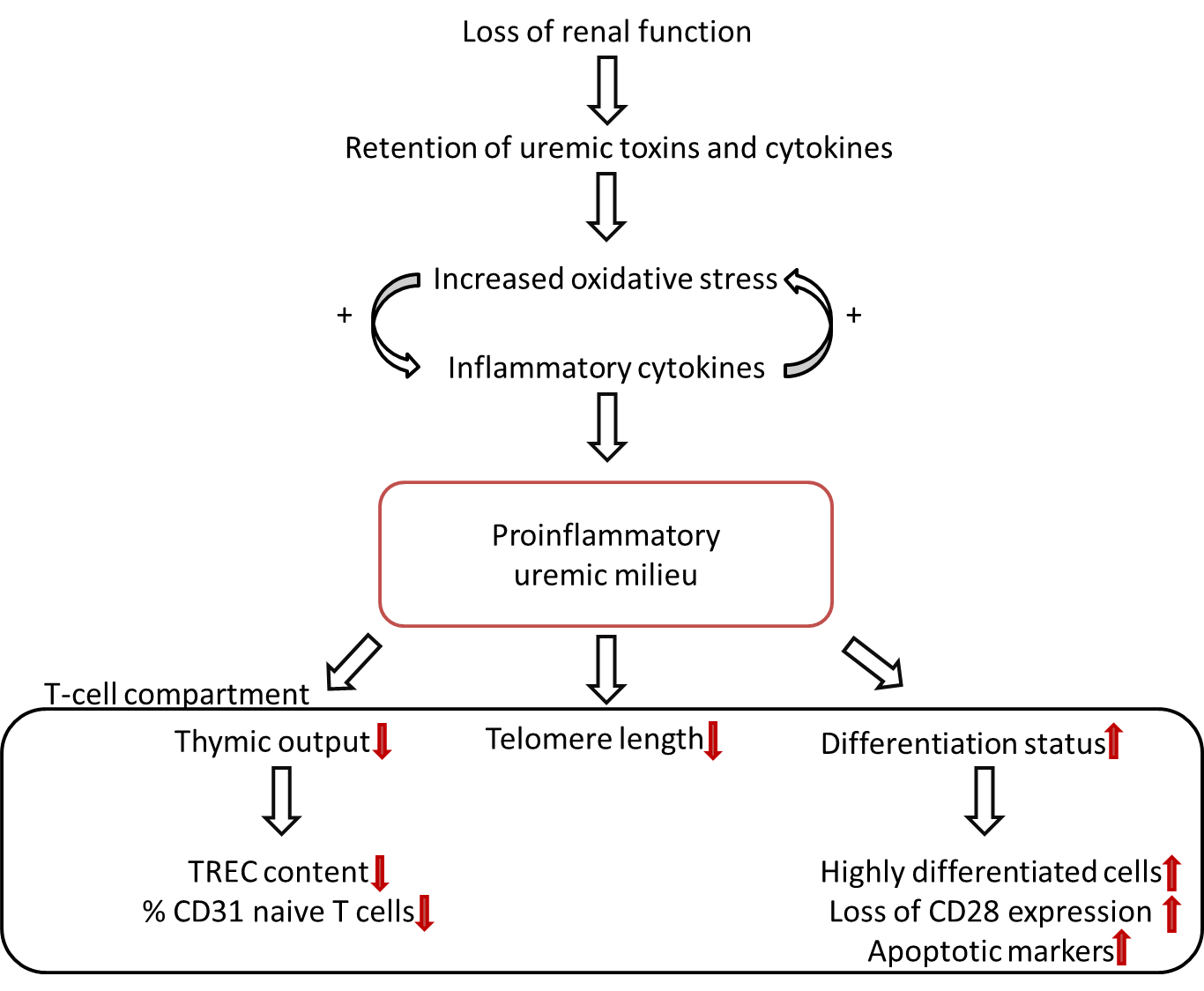
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**Figure 1 Schematic overview of the effects on the T-cell compartment caused by the uremia-induced pro-inflammatory milieu in end-stage renal disease patients.** Loss in renal function creates a pro-inflammatory milieu by the retention of uremic toxins and cytokines which increases oxidative stress and the production of inflammatory cytokines. This pro-inflammatory uremic milieu is associated with premature T-cell ageing, which results in defective T-cell immunity. End-stage renal disease (ESRD) patients have a lower thymic output of naïve T cells which can be measured by the TCR excision circles (TREC) content and the percentage of CD31-expressing naïve T cells. Furthermore, ESRD patients have an expanded population of highly differentiated T cells with a loss in CD28 expression and an increase in apoptotic markers. Moreover, these expanded T cells have a high proliferative history causing a decline in telomere length which can be measured by the relative telomere length analysis.