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Klinefelter syndrome, cardiovascular system and thromboembolic disease. Review of literature and clinical perspectives

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Abstract

Klinefelter Syndrome (KS) is the most frequently occurring sex chromosomal aberration in males, with an incidence of about 1 in 500 to 700 newborns. Data acquired from large registry-based studies revealed an increase in mortality rates among KS patients when compared with mortality rates among the general population. Among all causes of death, metabolic, cardiovascular and hemostatic complication seem to play a pivotal role. KS is associated, as are other chromosomal pathologies and genetic diseases, with cardiac congenital anomalies that contribute to the increase in mortality. Aim of the current study was to systematically review the relationships between KS and the cardiovascular system and hemostatic balance. In summary, patients with KS display an increased cardiovascular risk profile, characterized by increased prevalence of metabolic abnormalities including DM, dyslipidemia and alterations in biomarkers of cardiovascular disease. KS does not, however, appear to be associated with arterial hypertension. Moreover, KS patients are characterized by subclinical abnormalities in LV systolic and diastolic function and endothelial function, which, when associated with chronotropic incompetence may led to reduced cardiopulmonary performance. KS patients appear to be at a higher risk for cardiovascular disease, attributing to an increased risk of thromboembolic events with a high prevalence of recurrent venous ulcers, venous insufficiency, recurrent venous and arterial thromboembolism with higher risk of deep venous thrombosis or pulmonary embolism. It appears that cardiovascular involvement in KS is mainly due to chromosomal abnormalities rather than solely on low serum testosterone levels.

On the basis of evidence acquisition and authors’ own experience, a flow-chart addressing the management of cardiovascular function and prognosis of KS patients has been developed for clinical use.

1. Introduction

Klinefelter syndrome (KS) is the most common abnormality of sex chromosomes (47, XXY or a mosaic karyotype) and is characterized by hypergonadotropic hypogonadism [3]. Data suggests incidence of KS to be as high as 1/660 of newborns. [1, 2] Despite its first mention being 70 years ago [3], little data is available with regard to the morbidity and mortality of KS. Data from recent large registry-based studies [4-8] displayed an increase in mortality in KS patients when compared to the general population. Interestingly, mortality was specifically increased by concomitant cardiovascular diseases: KS was associated with a significant increase in mortality risk by 40% (Hazard ratio (HR) for all-cause mortality = 1.40; HR cardiovascular mortality = 1.41). However, it should be acknowledged that these studies were only based on those cases of KS that have been clinically diagnosed; thus undiagnosed KS cases may underestimate cardiac mortality.

Indeed, several reports suggest that KS is associated with a higher cardiovascular risk profile, subclinical cardiovascular abnormalities and impaired exercise performance. Surprisingly, it appears that KS patients are at lower risk for ischemic heart disease, although other cardiovascular events are more common in patients with KS [7].

Aim of this work was to systematically review the relationships among KS and the cardiovascular system, and alterations of hemostasis and thrombosis. We searched Medline for articles published in any language until July, 28 2015, with the following keywords: “Klinefelter syndrome”, “cardiovascular”, “heart”, “congenital abnormalities”, “diabetes mellitus”, “metabolic syndrome”, “hemostasis and thrombosis”, “platelet hyperaggregability”. Accordingly, we identified 90 articles.

2. Cardiovascular risk profile in Klinefelter Syndrome

The increased cardiovascular mortality observed in KS should, in theory, point to a higher prevalence of cardio-metabolic risk factors in these subjects. However, little information is available with regard to the prevalence of traditional cardiovascular risk factors in KS, or to the presence of subclinical cardiovascular involvement.

2a. Metabolic Syndrome

Few works, aimed at investigating the prevalence of metabolic syndrome (MS) in subjects with KS, showed a high prevalence of this pre-clinical condition in KS **(Table 1)**. In particular, Bojesen et al.[9] compared 70 KS subjects with a control population and showed a striking increase in MS prevalence in KS (42% in KS *vs.* 10% in controls).

Ishikawa et al. [10] found a prevalence of 34% of MS in 60 KS patients, confirming previous observations. Recently, Pasquali et al. [11] showed a prevalence of 50% in 69 KS subjects, compared with 10% in the control group, and a MS prevalence of 28% in a population of non-KS, testosterone-treated, hypogonadotropic hypogonadic subjects. Moreover, despite the limitations in terms of study size, in prepubertal adolescents with KS Bardsley et al. [12] showed an increased prevalence of MS (about 7%) compared with healthy age-matched subjects. Even for a similar BMI, infants and adolescents (4-18 y) with KS have a higher level of body fat, and especially of truncal fat (BFtr) with a reduction in lean mass, than the general population[13]. Bojesen et al.[9] found that the strongest predictor of MS was adiposity, especially BFtr. In a multivariate analyses BFtr was the independent variable with the most significant impact on both metabolic syndrome and measures of insulin sensitivity. Interestingly, when controlling for BFtr, the impact of hypogonadism in the presence of the MS or not and on insulin sensitivity disappeared, supporting the hypothesis that measures of insulin resistance, hepatic glucose output, and insulin secretion were not dependent on sex hormone levels after controlling for upper body obesity. The authors[14] suggested that a vicious cycle might ensue in KS, with hypogonadism influencing body composition, causing an increase in body fat (especially intra-abdominal fat), subsequently deteriorating carbohydrate metabolism, causing insulin resistance which further aggravates the hypogonadism via a direct effect on Leydig cell production of residual testosterone.

Although the relatively small sample size and the non-mechanistic nature of the studies, these data support the hypothesis that the increased visceral fat precedes the hypogonadism and that MS may be associated with KS independent of the hypogonadism. In addition, testosterone therapy does not appear to change the prevalence of MS[9, 11], nor improve indices of insulin resistance (IR). Interestingly, MS is closely associated with a low-grade chronic inflammatory status characterised by abnormal cytokine production, which activates a network of inflammatory signalling pathways. Overproduction of CCL2 is associated with insulin resistance. Rotondi et al [15] showed significantly higher serum levels of CCL2 in KS compared with controls. On the contrary, no significant differences in serum CXCL10 and adiponectin were observed between the two groups. In vitro studies have shown that testosterone exerts a powerful anti-inflammatory effect, as assessed by its ability to reduce the secretion of several cytokines and chemokines including CCL2. However, acute testosterone deprivation in healthy men leads to an increase in serum CCL2 levels, which is not reversed by restoration of physiological circulating concentrations of testosterone. Furthermore, the differences in the response to testosterone replacement therapy in KS could be dependent upon androgen receptor polymorphism[16-18]. These results suggest that, in addition to hormonal factors, a genetic predisposition, possibly mediated through macrophage infiltration into adipose tissue, is involved in the development of MS in KS[15].

2b. Diabetes

Since Mirouze and colleagues coined the term "*Prediabetes* in KS" [19] in 1966, most studies reported an increased incidence of diabetes mellitus (DM) in KS [5, 7, 19-26]. In large registry-based studies, Bojesen [5] and Swerdlow [7], taking into account cause-specific mortality ratios, showed a relative risk (RR) of DM of 1.64 and 7.07 respectively. Furthermore, KS and DM are associated with increased mortality [7]. Of note, replacement testosterone therapy does not seem to affect the prevalence and incidence of DM in KS. (**Table 1**) Unfortunately, the data on testosterone replacement in KS are extremely heterogeneous in modality, length of treatment and preparation used. Based on current evidence, it cannot be excluded that a lack of reversibility is related to inadequate regimen schemes, such as those producing repeated peaks and nadirs, as with some old formulation of injectable testosterone esters, suboptimal dosing secondary to a low absorption or an excessive delay in commencing replacement therapy leading to irreversible changes. The most recent meta-analysis on the cardiovascular safety of testosterone replacement in the general population[27], failed to identify a difference in events associated with the type of preparations used. However, society guidelines suggest transdermal preparations or long-acting injectable T undecanoate to reduce the risk of excessive hematocrit increase[28, 29].

2c. Dyslipidemia

Dyslipidemia has been reported in KS, consisting in high levels of total and LDL cholesterol as well as tryglicerides [30]. Bojesen et al., comparing testosterone-treated and untreated KS, showed lower LDL and total cholesterol levels in the Testosterone-treated group[9]. However, these data were not confirmed by Pasquali et al. [11] **(Table 1)**.

2d. C-reactive protein

Another biomarker measured in KS patients is C-reactive protein (CRP), a well-known inflammatory protein that predicts cardiovascular outcome [31]. In KS, CRP levels are increased at baseline [9, 32] and significantly reduced in the Testosterone-treated group[9].

2e. Endothelial progenitor cells

It has been demonstrated that reduced circulating endothelial progenitor cells (EPCs) are independent predictors of atherosclerotic progression and morbidity/mortality due to cardiovascular disease [33]. Specifically, the concentration and the migratory activity of EPCs inversely correlates with risk factors for coronary artery disease [34, 35]. Di Mambro et al. demonstrated a reduced number of EPCs in 68 KS subjects compared to age-matched controls and hypogonadal patients, independent of testosterone levels and of the presence/absence of other cardiovascular risk factors [36, 37]. Interestingly, testosterone replacement therapy exerted no effect on EPCs number, differently from what was observed in normal, testosterone-treated subjects [38]. Congruent with this observation, Ru et al.[39] showed that in KS subjects testosterone levels were not correlated with the number of EPCs **(Table 1)**. Given the growing interest of the scientific community in the study of EPCs [40-44], further studies are needed to explain the relationship between EPCs and KS.

2f. Leptin and Adiponectin

An intriguing biomarker studied in KS is leptin, which provides an afferent signal in a negative-feedback loop regulating the size of adipose tissue mass. Leptin is produced by adipocytes, and it is directly related to body-fat mass [45]. In KS, increased levels of leptin are demonstrated [9] with no difference in the Testosterone-treated group [9].

Interestingly, it seems that patients with KS are somehow protected by arterial hypertension (AH). A possible explanation for this finding may involve adiponectin physiology. Low levels of this hormone are indeed associated with systemic arterial hypertension, DM and coronary artery disease [46-48]. Although decreased levels of adiponectin in the general population characterize MS, KS subjects with MS display normal levels of this adiponectin [9] and this may prevent the development of AH in KS. Of note, in KS hypogonadism is relative rather than absolute. The non-suppressed level of adiponectin may therefore be the result of the opposing effect of (subnormal) testosterone levels and obesity.

Taken together, patients with KS display an impaired cardiovascular risk profile characterized by increased prevalence of metabolic abnormalities including DM, dyslipidemia and alteration in biomarkers of cardiovascular disease. However, KS does not appear associated with arterial hypertension.

3. Structural and functional cardiovascular abnormalities in Klinefelter Syndrome

Resting EKG characteristics in KS have been recently studied by Jørgensen et al[49]. These authors found a shorter QTc-interval in KS compared with controls. However, QTc was shortest among testosterone-treated males with KS, while untreated and hypogonadal KS had intervals comparable to controls. No mutations of genes related to short QT syndrome were found. These results suggest that genes on the X chromosome could be involved in the regulation of the QTc interval and that testosterone treatment significantly modulates this mechanism. Recently, EXAKT trial suggests that cardiac rhythmogenic stability, expressed as 12-lead EKG QTc time, was markedly altered in KS patients[50]. In this cross-sectional prospective project involving 132 KS patients, authors demonstrated that QTc time was significantly shorter in those patients showing higher levels of differentially expressed genes (DEGs). Pathologically short QTc times (< 370 ms) were observed in 11 KS patients but in none of the controls. In particular, the effect was even more pronounced in those men with a paternal origin of the supernumerary X chromosome. Moreover, serum T levels were not associated with QTc times[50]. Karagoz et al.[51] reported a case of a sinus node dysfunction requiring permanent pacemaker implantation in a 22- year-old man with KS.

Few pioneering reports aimed at assessing left ventricular (LV) structure in KS were performed by Fricke et al. [52, 53]. In these studies, a prevalence of 55% of mitral prolapse (MVP) was found in 22 patients with KS. On the contrary, despite two case reports confirmed the presence of mitral valve prolapse in KS[54, 55], two more recent large studies [11, 14] (25 and 69 patients respectively) did not confirm this finding. Andersen et al [14] found only subclinical alteration of the LV systolic function (reduction in LV strain and strain rate) with normal left ventricular ejection fraction in 25 KS subjects. A subgroup analysis showed that only KS subjects with MS ~~showed~~ displayed such alteration in that no differences between T-treated and untreated patients were found (median duration therapy of 9.5 years). The correlation between strain/Doppler indices of systolic function and fasting triglyceride and truncal body fat led the authors to speculate that myocardial systolic function impairment was strictly related with MS rather than to KS itself. To support this hypothesis, this pattern is commonly found in patients with obesity and MS, and appears linked to insulin resistance [56, 57].

Pasquali et al. [11] showed no significant difference in LV structure in 69 KS patients compared to controls, nor evidence of MVP. In the same study, no significant alterations of LV systolic function were reported, although strain analysis was not performed [11]. With regard to diastolic function, Andersen et al [14] showed a 20% prevalence (5/25) of diastolic dysfunction in KS patients. In particular, in a multiple regression analyses considering measurements of mitral inflow, peak E (early diastolic filling) and A (late diastolic filling), velocities ratio (E/A) (but not E and early diastolic annular velocity ratio (E/E')) significantly correlated with truncal body fat. Accordingly, Pasquali et al. [11] reported a significant prolongation of isovolumic relaxation time and mitral deceleration time, decreased E/A ratio and pulmonary vein velocities consistent with mild diastolic dysfunction; with no differences observed between treated and untreated KS patients. Notably, patients with secondary hypogonadism on testosterone therapy did not display normal cardiovascular parameters (**Table 2**).

With regard to cardiopulmonary exercise performance, Bojesen et al. [9] showed a reduced peak oxygen uptake (VO_2 max) in 70 KS patients, with no difference between treated and untreated subjects. In a multivariate analysis, VO_2 max was negatively correlated to body truncal fat, diagnosis of KS, 17®-estradiol, and age but positively to the intermuscolar adipose tissue-free skeletal mass. KS *per se* was the strongest (negative) predictor of VO_2 max, followed by skeletal muscolar mass. Pasquali et al. [11, 58]observed an impaired cardiopulmonary performance and exercise capacity in KS reporting a marked reduction of VO_2 peak and workload both at peak exercise (-34% vs. controls) and anaerobic threshold (-24% vs. control) compared to controls. Interestingly, KS displayed a remarkably increased prevalence of chronotropic incompetence (CI) defined as a lower proportion of predicted maximum Heart Rate (HR) (78 vs 91 %, p<.05) and a lower increase in HR from baseline to exercise peak (74 vs 91 bpm, p<.01) **(Table 2)**. CI is a common finding in several cardiovascular diseases [58], produces exercise intolerance that greatly impact on quality of life; and is an independent predictor of major adverse cardiovascular events and overall mortality in asymptomatic population [59, 60].

Several studies reported the predictive role of carotid intima media thickness (cIMT), a surrogate marker of atherosclerotic disease, on future cardiovascular event. Reduced flow mediated dilation (FMD), briefly described as endothelium-dependent vasodilation assessed by measuring the maximum increase in brachial artery diameter during reactive hyperemia created by the inflation of a cuff (250 mm Hg for 5 min) placed on the right arm, has been considered as a predictor of cardiovascular disease, although its value for risk stratification is still debatable [61, 62]. Foresta et al.[63] comparing 92 KS subjects with controls, showed ~~a~~ reduced diameters of brachial, common carotid, common femoral arteries and abdominal aorta arteries. No difference between KS patients and control with regard to cIMT and FMD were found. On the other hand, KS patients enrolled in the study by Pasquali et al [11] exhibited a significant increase of cIMT **(Table 2)**. It should be highlighted that difference in cIMT are not clinical relevant, since in both studies, is lower than 0.9 mm[64].

Recent data suggest that the vasculature of the testis might be altered in animal models of KS[65]. Interestingly, an alteration in vascular density and flow is observed early in KS boys during pubertal development[66] and it has been correlated with progressive LH rise. Little is known on the microvascular status of other tissues, however, the increased frequency of autoimmune disorders in KS[67] suggests that other than hormonal mechanisms could also be involved in altering tissue perfusion.

In spite of the fact that KS is the second most frequent chromosome disease and that almost 15-20 % of all congenital cardiovascular disease (CCD) are related to chromosomal disease [68, 69], few data are available addressing the prevalence of congenital heart diseases in this population. Compared to the general population, Bojesen et al.[4] showed a significant increase in CCD risk (HR 4.71), in KS. Among 3550 KS subjects, Swerdlow et al [7] reported that CCD was the specific cause of mortality in 5 patients, (Standardized Mortality Ratio = 7.3). To the best of our knowledge, all cases of CCD in KS [68, 70-94] are fully reported in the Supplemental Data 1.

In summary, KS patients are characterized by subclinical abnormalities in LV subclinical systolic and diastolic function and endothelial function, which, together with chronotropic incompetence, may lead to impaired cardiopulmonary performance. Moreover, KS patients appear to be a higher risk of CCD.

4. **Thrombosis and hemostasis in Klinefelter Syndrome**

Data from large registry-based studies [4-7] show that KS subjects are at increased risk of thromboembolic events. The hypothesis of an imbalance between thrombosis and hemostasis is suggested by the high prevalence (7-13%) [95, 96] of recurrent venous ulcers in KS [97], which in turn might be due to a previous post-thrombotic syndrome. Vein insufficiency is more prevalent in KS (about 20%) than in the general population [98]. Mesenteric vein thrombosis and arterial ischemia/infarction [99] prevalences are moderately increased for KS [5-7, 55, 100]. Moreover, a higher risk of both recurrent venous and artery thromboembolism has been shown in KS, with an HR of 2.15. Campbell et al [95] found that the risk of deep venous thrombosis or pulmonary embolism was 5-20 times higher in KS than in normal males. Although excessive thromboembolic morbidity represents a significant burden in KS, no study has systematically explored the pathophysiological underpinnings of this phenomenon. However, despite scant available literature (most of data results from clinical cases or have a small sample size), some hypotheses maybe put forward: 1) vascular abnormalities and/or worse risk profile for venous thrombosis [101-104]; 2) abnormalities in fibrinolysis with increased plasma activity of plasminogen activator inhibitor-1 (PAI-1) [105-110]; 3) Increased activity of factor VIII [111, 112], 4) platelet hyperaggregability. [113, 114]. Recently, our group in an effort to evaluate platelet reactivity and the expression of platelet activation markers in KS has conducted a cross-sectional study. Twenty-three consecutive KS patients under testosterone replacement therapy have been included as case group and 46 age-matched healthy males recruited among hospital staff served as controls. We observed an increased platelet reactivity in KS [115]; 5) Deficit and inhibition of C and S protein [116-123]; 6) high levels of homocysteine associated with antithrombin III (AT-III) alterations [124] or other; 7) factor V Leiden alterations [125-127]. See Supplemental data 2 for details.

It is worth mentioning the role of testosterone replacement therapy in hemostasis. Although the direct and indirect physiological role of testosterone and androgens on the coagulation system is well known [128-134], there is currently no clear evidence about the impact of hormone replacement therapy on the risk of venous thromboembolism in patients with KS. Some case-reports showed an improvement of leg ulcers and laboratory parameters with replacement therapy [110, 111, 122, 123, 135] (see Supplemental data 2). On the other hand, some authors suggested a detrimental role of testosterone therapy on the hemostatic balance [136]. In the paper by Di Minno et al. [115], no correlation between increased platelet reactivity and testosterone and estradiol levels in KS subjects studied under testosterone replacement therapy[115] was found. However, only patients receiving hormonal replacement therapy were evaluated, thus limiting the study's conclusions.

Consequently, the role of testosterone replacement therapy in thromboembolic risk in KS patients is still unclear. Controlled studies are needed for attempting to find a definitive pathophysiological explanation for the thrombophilic alterations characterizing KS. It's important to emphasize that KS should be considered in the differential diagnosis of a male patient with non-healing ulcers of the lower extremities.

In summary, KS patients are characterized by an increased risk of thromboembolic events with high prevalence of recurrent venous ulcers, vein insufficiency, both recurrent venous and artery thromboembolism with higher risk of deep venous thrombosis or pulmonary embolism than general population. To date, there is no clear evidence of the impact of hormone replacement therapy on the risk of venous thromboembolism in patients with KS.

5. Are the cardiovascular abnormalities in Klinefelter Syndrome due to hypogonadism or to the syndrome itself?

To date, two main hypotheses might be put forward to explain the cardiovascular involvement in KS subjects: is the hypogonadism the main player responsible for cardiovascular involvement in KS or is the KS *per se* the culprit? In the following section, both hypotheses were briefly discussed.

Hypogonadism may play a pivotal role in the determination of some conditions including MS and dyslipidemia that, in turn, may impact on exercise capacity and overall cardiovacular status. However, the lack of evidence that testosterone replacement therapy might improve exercise capacity, skeletal muscle performance, insulin resistance in KS [11]at variance with data reported in the general population[137] does not support a prominent pathophysiological role of hypogonadism. In this complex scenario, it is worth pointing out that the clinical response to testosterone therapy is influenced by the polymorphism of the gene encoding for the X-linked androgen receptor gene, which is characterized by a certain number of CAG repeats (CAGn) (the length of the CAGn is inversely associated with androgen sensitivity) [18, 138]. A high number of CAGn is a common finding in KS genotype, and it may significately modulate the clinical response to testosterone therapy [18]. Bojesen et al. demonstrated an impact of the CAGn polymorphism on the phenotype of KS. In this study, involving 70 KS patients and 70 age-matched control subjects, they showed that although the number of CAG repeats was no different from controls, it did affect height, arm span, total cholesterol, haemoglobin and hematocrit within the KS cohort, but did not impact the effect of testosterone treatment in KS [139].

Pasquali et al[11] recently proposed that the chromosomal abnormality plays a major role in inducing cardiovascular phenotype of KS patients. In this study, the authors specifically studied a group of normal karyotype hypogonadal patients under adequate testosterone replacement therapy, who displayed a normalization of the cardiovascular abnormalities that did not occur in matched KS patients under similar replacement regimens.

Despite these studies not being specifically designed to provide mechanistic insight into the pathophysiology of the abnormalities found in KS, these observations suggest a complex interaction between chromosomal and hormonal factors (chromosomal abnormality is associated with clinical response to hormones) being the testosterone action on target tissues the actual deficient process.

KS might represent a natural human model of androgen deprivation. Given the known properties of testosterone on the cardiovascular system [140-144], it may be relevant to study these young subjects with regard to the cardiovascular function and determine the effects of a long-term testosterone deficiency/insensitivity.

In conclusion, it could be argued that cardiovascular involvement in KS is mainly due to chromosomal abnormalities rather than to low serum testosterone levels. However, the chromosomopathy maybe strictly related to the magnitude of the testosterone activity on the tissues.

In addition, an alteration of androgen pulses or release from the testis has been recently hypothesized[65]. An alteration of the release from the testes due to an impaired testicular vascular bed could be responsible either for lower circulating levels or impaired secretory rhythm.

6. Clinical implications

Patients affected by KS display an impaired metabolic risk profile characterized by an increased prevalence of MS and DM. This may lead to subclinical systolic and diastolic dysfunction and vascular abnormalities, which in turn might sustain the impaired cardiopulmonary performance.

In most studies, the subtle cardiovascular abnormalities were not reverted by testosterone replacement. It seems reasonable to consider, in the medical management of KS [13, 30, 145-149], a complete cardiovascular work-up in KS patients, in order to diagnose and correct preclinical and clinical abnormalities, with the aim of an overall reduction of the cardiovascular risk.

Specifically, if KS diagnosis is made during childhood, all patients should undergo a complete echocardiographic study to look for possible cardiac abnormalities. If the diagnosis of KS is made during adulthood, echocardiographic study should be focused on pre-clinical systolic and diastolic dysfunction. If no alterations are found, patients need follow up based on available risk-assessment [150, 151](**figure 1**).

Considering the risk of overlooking the underlying diagnosis of KS, we suggest a flow-chart to guide cardiologists to select the right patient to consider for endocrinologic consultation (**figure 2**).

Considering the unequivocal finding of an increased mortality of KS patients, mostly related to cardiovascular disease, more research is needed to characterize these alterations and to explain the underlying pathophysiological background.

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Table 1. Characterization and effects of testosterone replacement therapy on cardiovascular risk factors in Klinefelter Syndrome. (Rev: review of literature).

First author (year)[reference]	n. patients	Findings	Effect of Testosterone treatment (TT)
	Metabolic syndrome		
Bojesen (2006) [9]	71	42% KS vs 10% in controls	-
Ishikawa (2008) [10]	60	34%	-
Pasquali (2013)[11]	69	50% KS vs 10% in controls	No effect
Bardsley (2011) [12]	89	7% in young KS; 24% HOMA > 2.5	-
	Diabetes Mellitus		
Jackson (1966) [20]	Rev	12%	-
Becker (1966) [21]	50	10%	-
Ota (2002) [23]	895	6,5 % in Japan	No effect
Takeushi (1999) [22]	Rev	15%-50% in Western countries 3.9%-4.1% in Japan	-
Bojesen (2004) [5]	781	DM hazard ratio 1.64	-
Swerdlow (2005) [7]	3518	DM cause-specific mortality ratio 7.07; standardized mortality ratio :5.8; Hazard Ratio: 1,6	-
	Dyslipidemia		
Bojesen (2006) [9] Lanfranco (2004) [29] Bardsley (2011) [12]	71 Rev 89	Increased total cholesterol,, LDL cholesterol, Triglicerides and decreased levels of HDL.	Contrasting data on effect of TT on improving lipidic profile
	Hormones and biomarkers		
Bojesen (2006) [9] Host (2010) [31]	71 19 untreated, 20 treated	**CRP** levels increased at baseline compared to controls	Reduction in CRP levels
Bojesen (2006) [9]	71	Increased levels of **Leptin** at baseline compared to controls	No effect
Host (2010) [31] Pasquali (2013) [11]	19 untreated, 20 treated 69	KS with MS display normal levels of **adiponectin** compared to MS controls,	No effect
Di Mambro (2010) [35] Ru (2012) [38]	68 36	Reduced concentration of **EPCs** KS compared to age-matched controls and hypogonadal patients	No effect

Table 2. Morphological and functional assessment of the cardiovascular system.
CR: case report

First author (year)[reference]	N. of patients (KS vs CTRL)	Findings	Effect of Testosterone Treatment (TT)
Electrocardiography			
Jorgensen (2015) [48]	62 vs 62	QTc-interval shorter in KS than in controls shortest in testosterone-treated KS comparable to controls in untreated KS	
Zintzmann (2014)[49]	132 vs 100	Pathologically short QTc times (< 370 ms) were observed in 11 KS patients but in none of the controls. Effect was even more pronounced in those men with a paternal origin of the supernumerary X chromosome. Moreover, serum T levels were not associated with QTc times	
Echocardiography			
Fricke (1981) [52] Fricke (1984) [51] Murray (1976) [54] Ueki (2004) [53]	22 22 CR CR	Increased prevalence of mitral valve prolapse (55%)	-
Pasquali (2013) [11]	69 vs 48	No difference in LV architecture. Higher prevalence of mild diastolic dysfunction in KS compared with controls	no effect
Andersen (2008) [14]	25 vs 25	subclinical alteration of the LV systolic function (reduction in LV strain and strain rate). High prevalence of 20% of diastolic disfunction; in multiple regression analyses, E/A ratio (but not E/E' ratio) significantly correlated with truncal body fat	no effect
Cardiopulmonary Ecercise Test			
Pasquali (2013) [11]	69 vs 48	Marked reduction of VO_2 peak and workload Increased prevalence of Chronotropic Incompetence: 25 out of 48 (52%) vs no subjects in controls	no effect no effect
Bojesen (2006) [9]	70 vs 71	Reduced VO_2 uptake during exercise	no effect
Vascular assessment			
Foresta (2012) [62]	92 vs 50	Reduced diameters of brachial, common carotid, common femoral arteries and abdominal aorta arteries No difference cIMT and FMD	-
Pasquali (2013) [11]	69 vs 48	significant increase of carotid IMT	no effects

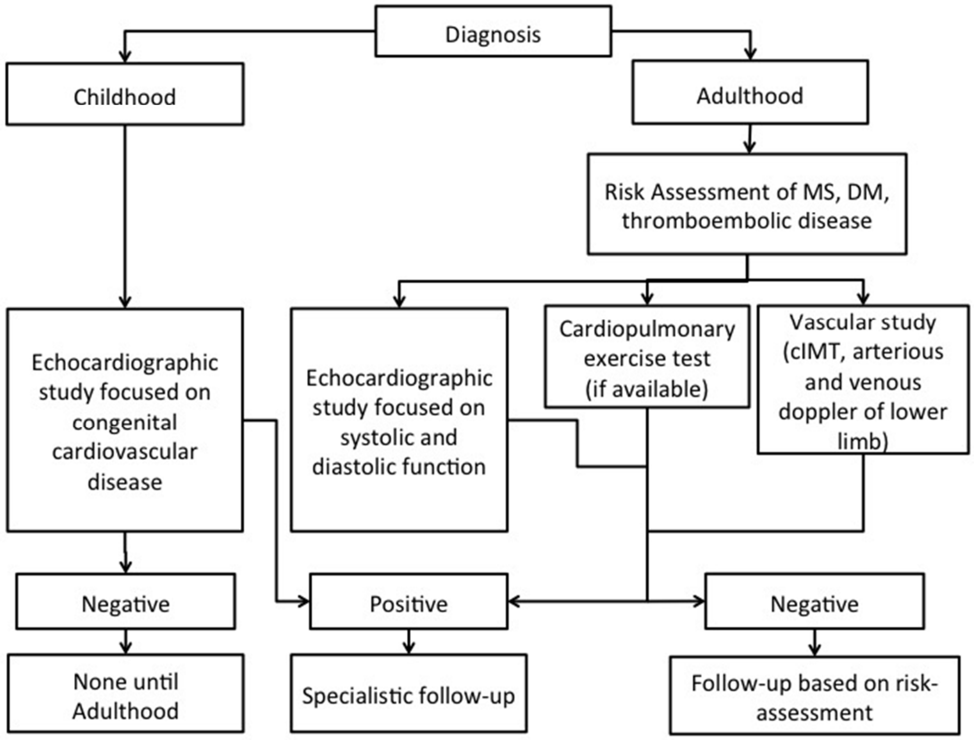

Figure 1. Suggested flow-chart for cardiovascular and metabolic assessment and follow-up in Klinfelter Syndrome
254x190mm (72 x 72 DPI)

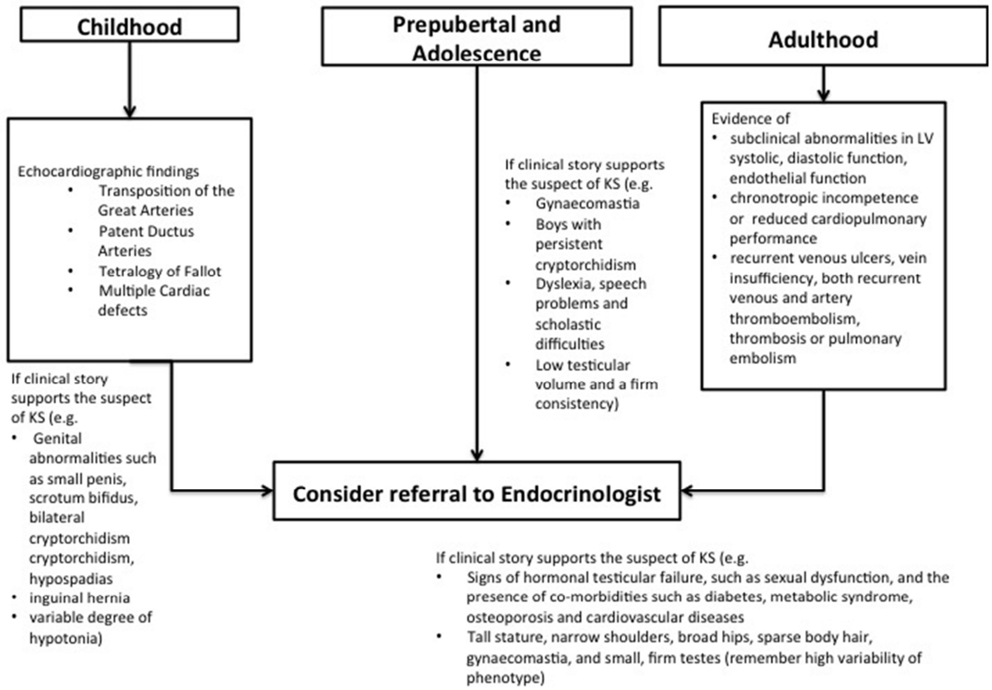

Figure 2. Suggested flow-chart for endocrinology referral by cardiologists diagnosing cardiovascular abnormalities potentially associated to KS
254x190mm (72 x 72 DPI)