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## **Primary aldosteronism in the elderly**

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2 **ABSTRACT**

3 **Context:** the clinical spectrum and knowledge of the molecular mechanisms underlying primary  
4 aldosteronism (PA), the most frequent form of endocrine hypertension, has evolved over the past  
5 years. In accordance with the Endocrine Society guideline and in light of the growing evidence  
6 showing adverse cardiovascular outcomes, it is expected that a progressively wider population of  
7 patients affected by hypertension will be screened for PA, including the elderly.

8 **Evidence Acquisition:** a systematic search of PubMed was undertaken for studies related to renin-  
9 angiotensin-aldosterone system (RAAS), primary aldosteronism and adrenal histopathology in the  
10 elderly population.

11 **Evidence synthesis:** several studies showed an age-dependent decrease in the activity of RAAS,  
12 together with a progressive decrease of the aldosterone response to sodium intake, particularly after  
13 the sixth decade of life. The positive correlation between age and serum aldosterone during liberal  
14 sodium intake over serum aldosterone during sodium restriction is paralleled by histological changes  
15 in adrenal aldosterone synthase (CYP11B2) expression patterns. Immunohistochemical studies  
16 showed a progressive loss of the continuous expression of CYP11B2 in adrenal zona glomerulosa  
17 with ageing and a concomitant increase of aldosterone-producing cell clusters, which might be  
18 responsible for a relatively autonomous aldosterone production. Additionally, following PA  
19 confirmation and subtype diagnosis, older age is correlated with a lower benefit after adrenalectomy  
20 for unilateral PA.

21 **Conclusions:** accumulating evidence suggests that RAAS physiology and regulation show age-  
22 related changes. Further studies may investigate to what extent these variations might affect the  
23 diagnostic work-up of patients affected by PA.

## 24 **Introduction**

25 Primary aldosteronism (PA) is a condition of inappropriate aldosterone production for renin levels  
26 and sodium status (1). Over the last two decades, clinical studies have provided evidence that PA is  
27 the most frequent cause of secondary hypertension (2-4) and is associated with a higher occurrence  
28 of cardiovascular and renal damage and metabolic complications compared with essential  
29 hypertension (5,6). The Endocrine Society (ES) guideline recommends screening of patients with  
30 hypertension and other risk conditions, resulting in the potential screening of at least 50% of  
31 patients with hypertension (1). Despite the low application of the Guideline, even in developed  
32 countries (7), it is expected that a progressively wider population of patients with hypertension will  
33 be screened. The Guideline does not give specific indications for a different strategy for screening  
34 patients according to age. However, many physicians tend to study the younger rather than older  
35 patients more extensively.

36 In this manuscript we review the available data on variations of aldosterone production with age,  
37 the pathophysiological changes in aldosterone regulation and in adrenal pathology. We will also  
38 discuss the appropriate strategies for diagnosis and management of PA in elderly patients.

## 39 **Genetic alterations in sporadic and familial PA**

40 PA is more frequently a sporadic condition, comprising unilateral and bilateral forms. Unilateral  
41 forms comprise aldosterone-producing adenoma (APA) and unilateral diffuse hyperplasia or  
42 hyperplasia with multiple nodules and account for 30-40% of cases of PA. Bilateral PA also known  
43 as idiopathic hyperaldosteronism or bilateral adrenal hyperplasia, is the most frequent form  
44 accounting for the remaining sporadic cases. Familial hyperaldosteronism (FH) is relatively rare  
45 accounting for less than 5% of cases (8). After the demonstration in 1992 that FH type 1 (also  
46 known as glucocorticoid-remediable aldosteronism) was due to a recombination between *CYP11B1*  
47 and *CYP11B2* genes resulting in a chimeric gene regulated by adrenocorticotrophic hormone  
48 (ACTH) encoding a hybrid enzyme able to produce aldosterone (9), it wasn't until after 2011 that

49 CLCN2 (10), KCNJ5 (11), and CACNA1H (12), were demonstrated to be the genes involved in the  
50 pathogenesis of FH2-4. A further gene, *CACNA1D* (13), was shown to be responsible for a genetic  
51 but not familial condition named PASNA (primary aldosteronism, seizures and neurological  
52 abnormalities). In FH2-4 and in the PASNA syndrome, aldosterone hyperproduction is due to an  
53 alteration of the function of ion channels, resulting in an increase of intracellular calcium in zona  
54 glomerulosa cells of the adrenal cortex, that activate CYP11B2 transcription and aldosterone  
55 production (14,15). Somatic mutations in these genes and in the *ATP1A1* and *ATP2B3* were shown  
56 to be responsible for the dysregulated aldosterone production in sporadic APAs (14-18).

### 57 **Changes in the adrenal *zona glomerulosa* with aging**

58 The availability of specific monoclonal CYP11B2 and CYP11B1 antibodies allowed the  
59 understanding of the histological structures involved in aldosterone production (19) and the study of  
60 its changes during aging and in different pathological states (20,21). The use of these specific  
61 CYP11B2 antibodies allowed the identification of small, mainly subcapsular, nodules of CYP11B2  
62 expression, usually referred to as aldosterone-producing cell clusters (APCC) (22). APCC are  
63 present in both normal and pathologic adrenals (22-24); their presence in the adjacent cortex to an  
64 APA indicates that aldosterone production in these cells is not suppressed by the excessive  
65 aldosterone produced by the APA and therefore they could represent a source of potentially  
66 inappropriate or dysregulate aldosterone production in the adrenals (20). The exact role and  
67 function of these structures is unknown: for example, they are associated with a lower lateralisation  
68 index at adrenal vein sampling (AVS) and lower prevalence of contralateral suppression of  
69 aldosterone production, indicating that their presence may be bilateral in these patients operated for  
70 unilateral PA (25). Furthermore, APCC may also affect the results of ACTH stimulation during  
71 AVS (26). However, the presence of APCC was not associated with absent biochemical success,  
72 that is persistence of PA, after adrenalectomy (24). It was also demonstrated that in APCC from  
73 normal adrenals from kidney donors may carry somatic mutations in aldosterone driver genes such

74 as *CACNA1D*, *ATP1A1* and *ATP2B3* (27), genes that are also found mutated in sporadic APAs (16).  
75 In APAs the most frequently mutated gene is usually reported as *KCNJ5* (which is very rarely  
76 mutated in APCC) in contrast to APCC which show the highest incidence of mutations in  
77 *CACNA1D* (28). In patients with bilateral PA, APCC are more often present and increased in  
78 number and are more frequently mutated (29). Putative transitional structures with similarities to  
79 both APCC and APA have also been shown (30) and these transitional lesions, as well as APCC,  
80 are able to produce and accumulate both aldosterone and 18-oxocortisol, whose secretion is  
81 increased in many APAs (31). Further clues on the potential role of APCC as precursor lesions of  
82 APAs have been provided by the *in situ* metabolic phenotypes of APCC and APA in adrenals  
83 removed for unilateral PA (32): the authors identified 2 subgroups of APCC, one with specific  
84 distribution patterns of metabolites closely resembling those in APA and a different subgroup with  
85 a metabolic phenotype highly distinct from APA (32). Interestingly, all APCC within an adrenal  
86 displayed the same metabolite pattern (32).

87 Overall, these findings suggest that APCC could be involved in the pathogenesis of both unilateral  
88 PA, as precursors of APAs, and of bilateral PA as contributors of the dysregulated aldosterone  
89 production. The reason for the absence of mutations in *KCNJ5* in APCC may be related to the high  
90 expression of *KCNJ5* in APCC which may be incompatible with the presence of *KCNJ5* mutations  
91 which are associated with high cell toxicity (33). This is in contrast to the relatively lower  
92 expression levels of *KCNJ5* in APA with *KCNJ5* mutations compared with APA (33) carrying  
93 other mutations and the adjacent zona glomerulosa layer (33,34).

94 In young subjects, *CYP11B2* is expressed as a continuous pattern in the *zona glomerulosa* (23).  
95 With aging there is a progressive loss of the continuous expression of *CYP11B2* and a concomitant  
96 increase of APCC in the outer layer of the adrenal cortex (20,27) (Figure 1). The result of these age-  
97 related changes is that in older people, *CYP11B2* expression is mainly localized in the APCC  
98 structures whereas in the young, *CYP11B2* is expressed in a continuous layer in the *zona*  
99 *glomerulosa* under the capsule (20,27). These changes in the pattern of *CYP11B2* expression with

100 aging could explain in part the different production and regulation of aldosterone both under basal  
101 and stimulated conditions.

## 102 **Changes of aldosterone secretion with aging**

103 An age-dependent decrease in the activity of the renin-angiotensin-aldosterone system (RAAS) has  
104 been observed in normal subjects (35,36), independent from the status of sodium repletion. The  
105 reduction of basal and sodium depletion-stimulated renin production becomes evident after the sixth  
106 decade of life (35) (Figure 1). The concomitant decrease of aldosterone levels appears to reflect a  
107 decline of the angiotensin II stimulus rather than an alteration of *zona glomerulosa* function,  
108 because the response of aldosterone to ACTH stimulus is unchanged with aging (35). The lower  
109 RAAS activity is attributed to the reduction of renin, which in turn diminishes plasma renin activity,  
110 rather than angiotensinogen production (37). The decrease of renin levels with age is probably due  
111 to a deterioration of kidney function and has been used by many clinicians as the basis for the  
112 choice of anti-hypertensive therapy. For example, the NICE guideline 2019 for diagnosis and  
113 management of hypertension in adults (38) suggests the treatment of patients with hypertension  
114 aged more than 55 years with calcium channel blockers or thiazide diuretics, two classes of drugs  
115 that are more efficient in patients with hypertension and a low-renin profile (39,40). Aldosterone  
116 levels remain unchanged (23) or tend to decrease with aging (41) but less than renin levels. This is  
117 probably attributable to the reduced potassium secretion which parallels the decline in kidney  
118 function with aging and the subsequent stimulatory effects of potassium on aldosterone secretion.  
119 Between the fourth and the eighth decade of life there is a progressive lowering of the glomerular  
120 filtration rate (around 1 ml/min/year) and of the renal blood flow (42). Also, distal tubular function  
121 progressively declines and concomitantly, the ability to eliminate potassium, resulting in  
122 susceptibility to hyperkalaemia in the elderly (43). The potassium retention stimulates aldosterone  
123 production to maintain normokalemia: this results in an increase of the aldosterone-renin ratio  
124 (ARR) with age (23,41). This pattern of increased ARR with aging results in a progressively higher

125 number of patients with essential hypertension but with a high ARR in older patients (44), in a  
126 study from 0% in patients with hypertension aged less than 30 years to 21% in patients of 60 years  
127 or older (44). These observations may have an impact on the interpretation of screening and  
128 confirmatory/exclusion tests in older patients (41,44,45) (Figure 1).

129 Another aspect that should be considered is the progressive change of the aldosterone response to  
130 sodium intake with aging. To explore this parameter, researchers used the ratio between the serum  
131 aldosterone during liberal sodium intake and the serum aldosterone during sodium restriction  
132 (SASSI, Sodium-modulated Aldosterone Suppression-to-Stimulation Index) (46): higher values of  
133 SASSI indicate abnormal aldosterone regulation. A higher SASSI is associated with a reduced  
134 glomerular filtration rate and decreased renal plasma flow and therefore with the decline of kidney  
135 function (47) and is also associated with the severity of metabolic syndrome (46). Furthermore, a  
136 higher SASSI was associated with a higher Framingham risk score (47). In patients with  
137 hypertension a sharp decrease of the aldosterone response to angiotensin II was observed with aging  
138 in females but not in males (48). In another study a blunted renal plasma flow response to  
139 angiotensin II was observed with aging independent of sex and this response was inversely  
140 correlated with SASSI (47).

141 A subsequent study by Nanba et al., provided a potential pathophysiological link between the  
142 adrenal histopathological changes of CYP11B2 expression with aging and the progressive  
143 dysregulation of the aldosterone secretion (23). They observed a negative correlation of the area  
144 expressing CYP11B2 with increasing age but a positive correlation of APCC area with aging (23).  
145 These findings suggest a switch from a prevalent CYP11B2 expression in the whole *zona*  
146 *glomerulosa* to a prevalent CYP11B2 expression in APCC with aging (23) (Figure 1). In the same  
147 study the authors investigated the plasma renin activity and aldosterone levels in a group of 677  
148 subjects with normal blood pressure or hypertension stage I (49), under different conditions and  
149 correlated these parameters with age. In this large cohort, plasma renin activity progressively  
150 declined with age, whereas plasma and urinary aldosterone levels remained unchanged (23).

151 Therefore, ARR was significantly correlated with age, even after correction for confounding  
152 factors, including sodium excretion (23). Finally, SASSI was also positively correlated with age,  
153 indicating that the accumulation of APCC with age may cause an increased non-suppressible  
154 aldosterone secretion but in turn, the suppression of the normal *zona glomerulosa* is associated with  
155 an impaired aldosterone production under physiological stimuli such as sodium restriction (23).

### 156 **Diagnosis of primary aldosteronism in the elderly**

157 The ES guideline recommends a three-step procedure for the diagnosis of PA (1). This comprises  
158 screening, confirmation and subtype diagnosis (1). Subgroups of patients with a high prevalence of  
159 PA should be tested using the ARR: groups of patients with hypertension and at high risk for PA  
160 comprise patients with blood pressure higher than 150/100 mmHg including resistant hypertension,  
161 patients with spontaneous or diuretic induced hypokalemia, or with an adrenal mass (1). However,  
162 there are no specific recommendations for elderly patients and if the ARR cut-offs should be  
163 modified in this subgroup. It should be noted that hypertension is a highly prevalent condition in the  
164 elderly, with a prevalence of 50-70% in Western countries (50). It is expected that more than 30%  
165 of these patients would display an increased ARR, thus requiring a very high number of  
166 confirmatory/exclusion tests and potentially a high number of AVS if surgery is considered feasible  
167 for these patients. Therefore, it could be reasonable to restrict the screening with ARR to patients  
168 with a particularly florid phenotype (and therefore with an expected higher benefit from the  
169 diagnosis), such as resistant hypertension and or marked hypokalemia, or to consider the ARR just  
170 as a potential indication for the therapy and restrict the continuation of the diagnostic work-up only  
171 to those with the florid phenotype. Whatever is the choice, some specific features of the diagnosis  
172 of PA in elderly patients with hypertension should be acknowledged.

173 In older patients the ARR increases and thus the number of false positive results for screening can  
174 be high. In fact, children display a relatively low ARR cut-off compared with adults (51), and in  
175 adults, the ideal cut-off progressively increases (41). For example, the cut-off that has a specificity

176 of 0.82 in patients aged less than 40, displays a specificity of only 0.42 in patients older than 60  
177 years (41).

178 Patients with a positive ARR could be empirically treated with a mineralocorticoid receptor  
179 antagonist (MRA) or proceed to confirmatory/exclusion testing. If clinicians choose this option,  
180 they should take into account the risks of volume expansion under sodium loading in elderly  
181 patients in whom renal and cardiac function is more often impaired. In risk patients, the captopril  
182 challenge test may be the test of choice. In this case, clinicians should be aware that a recent study  
183 challenged the cut-off suggested by the Endocrine Society guideline and suggested that absolute  
184 aldosterone levels, rather than the percentage reduction, should be used to confirm/exclude the  
185 diagnosis of PA (52).

186 In patients with a positive confirmatory test, the subsequent subtype diagnosis should be considered  
187 case-by-case. Computed tomography (CT) scanning is used also to rule out the rare but potentially  
188 fatal aldosterone-producing carcinoma. By contrast, AVS should be considered in carefully selected  
189 patients. It should be performed only in patients not at risk of contrast-induced nephropathy that are  
190 potentially candidate for surgery, which can be contraindicated in many cases for concomitant  
191 illnesses. Furthermore, clinicians should consider that the proportion of patients with unilateral  
192 disease in the elderly may be lower than in the young and that age, duration of hypertension and  
193 reduced kidney function are correlated with a lower benefit after adrenalectomy as shown by the  
194 PASO study (53). Older age was also a negative predictor of clinical success in patients with PA  
195 adrenalectomized on the basis of the CT scanning findings alone, without undergoing AVS (54).  
196 Therefore, it is unlikely that an elderly patient with PA can achieve complete clinical cure with  
197 adrenalectomy. It seems reasonable to restrict AVS only to patients with a florid phenotype,  
198 otherwise healthy, with a short duration of hypertension and motivated for surgery or to patients  
199 with resistant hypertension in which MRAs are contraindicated or not tolerated. A particular  
200 subgroup is that of patients with hypertension and incidentally-discovered adrenal mass, which tend  
201 to increase in prevalence in the elderly. In these patients, when ARR is elevated, if adrenal surgery

202 is considered feasible by the clinician and desired by the patient, the complete diagnostic workup,  
203 including AVS, could be considered. In an area in which there is lack of studies investigating  
204 specifically the cost/benefit of diagnosis and treatment of PA, it is not possible to define a specific  
205 age cut-off to suggest a change in the diagnostic strategy: usually 65 years is the threshold to define  
206 elderly patients. However, different approaches should be considered in a 66 y.o. otherwise healthy  
207 female with a newly diagnosis of hypertension and hypokalemia and an 83 y.o. male with a long  
208 duration of hypertension and several cardiovascular comorbidities. It is reasonable to perform a  
209 personalized choice taking into account pro and cons of the different diagnostic steps and the  
210 expected benefit.

#### 211 **Therapy of primary aldosteronism in the elderly**

212 Patients with PA that undergo AVS and show lateralisation of aldosterone secretion (lateralisation  
213 index  $\geq 4$ ) should be adrenalectomized (1). Patients in whom surgery is contra-indicated or not  
214 desired by the patient should be treated with MRA (1). In the large Japanese registry of the JPAS  
215 study group showed that elderly patients with PA ( $\geq 65$  years) display worse kidney function and a  
216 higher rate of cardiovascular complications and diabetes (55). Furthermore, in agreement with the  
217 PASO study (53), complete clinical success was less common in elderly patients, despite a similar  
218 rate of complete biochemical cure (55). In these patients, the only independent predictor of  
219 complete clinical success was a lateralisation index higher than 4, a cut-off that is usually  
220 considered ideal to distinguish unilateral from bilateral PA (56). They also observed a higher rate of  
221 renal impairment, a more prominent deterioration of glomerular filtration rate and a higher rate of  
222 hyperkalemia in the elderly compared with non-elderly patients with PA after adrenalectomy (55).  
223 This is consistent with the reported worsening of kidney function after adrenalectomy in patients  
224 with PA (6), to which elderly patients may be particularly susceptible.

225 Patients with PA who are not candidates for surgery or do not display lateralisation at AVS should  
226 be treated with MRAs (1); when spironolactone is not tolerated and eplerenone not available,

227 treatment with amiloride should be considered. With the decline of kidney function in the elderly  
228 and with the reduction of plasma volume with MRA therapy (6), special care should be given to  
229 monitoring potassium levels, which can rapidly rise for concomitant deterioration of distal renal  
230 tubular function with aging and the consequent reduced ability to eliminate potassium (43). Since  
231 potassium is not the ideal indicator of MRA therapy efficacy and compliance, MRA-treated patients  
232 should periodically measure plasma renin activity or renin concentrations, since the increase of  
233 these parameters, indicating that renin is not suppressed anymore by the excess of aldosterone  
234 action, was associated with a lower rate of cardiovascular and renal complications in patients with  
235 PA during long-term follow-up (57,58).

## 236 **Conclusions**

237 Over the last years the clinical spectrum and the knowledge of the molecular mechanisms involved  
238 in the development of both sporadic and familial PA have substantially evolved. Accumulating  
239 evidence indicates that age-related modifications in RAAS pathophysiology might be more relevant  
240 than previously thought. In particular, recent studies have focused on the functional implications of  
241 APCCs, which carry somatic mutations in aldosterone-driver genes and might play an important  
242 role in both the development of bilateral PA and in the relatively autonomous aldosterone secretion  
243 in the elderly population. Further studies should be addressed at investigating whether these  
244 changes might influence the diagnostic work-up and the clinical management of patients affected by  
245 PA.

246

247 **Figure legend.** Simplified overview of RAAS pathophysiology in the elderly population. APCC,  
248 aldosterone producing cell clusters; ARR, aldosterone to renin ratio; CYP11B2, aldosterone  
249 synthase; PA, primary aldosteronism RAAS, renin-angiotensin-aldosterone system; SASSI,  
250 sodium-modulated aldosterone suppression-to-stimulation index; ZG, *zona glomerulosa*

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